Nov. 15 - 1909 —

MONTREAL DAILY STAR

Klotz
Coll.

DR. OSCAR KLOTZ, M'GILL PROFESSOR, GOES TO PITTSBURG

University Loses Still Another Professor of World-wide Repute for Research Work.

Dr. Oscar Klotz, assistant to Dr. Adami in Pathology in the Royal Victoria Hospital, is about to accept a very flattering offer from the University of Pittsburg. About the New Year Dr. Klotz will take the position of Professor of Pathology in Pittsburg. During his stay of seven years in Montreal in connection with McGill University and the Royal Victoria Hospital, Dr. Klotz has created for himself a European and American reputation for his original research work in many branches of his subject, but especially in the branch dealing with arterio-sclerosis, about which little is known, although it is an almost universal disease.

Dr. Klotz came to McGill as Governor's Fellow in Pathology. His reputation grew very quickly and the next year he took up work in connection with the Rockefeller Institute of Medical Research. In 1905, besides his work in McGill University as lecturer in pathology, he took the position of assistant pathologist at the Royal Victoria Hospital, which position he still holds.

In 1904 Dr. Klotz spent a year in research work in the pathological laboratories on the continent and especially in Germany, and again in 1908, six months in the same work.

He is now going to take the position of professor of pathology in the Univeristy of Pittsburg.

This university is in part privately endowed, but it is also supported by the State as being the Western University of Pennsylvania. The hospital facilities in Pittsburg will be very great for at the present time the authorities there are contemplating the building of a huge hospital at the cost of some $4,000,000.

During his stay in Montreal, Dr. Klotz has also been pathologist to the Maternity and Alexandra Hospitals. When Dr. Klotz received the offer from Pittsburg the hospital authorities here made every effort to retain him, giving him large promises of advancement, but when they realized the opportunities which Dr. Klotz would have in Pittsburg they felt it their duty to advise him to accept the position, although it will be almost impossible for them to fill his place.

Dr. Klotz has published a large number of important scentific papers, not only in American periodicals but also in those of Germany, and he has been frequently quoted as a leading authority on his subject by the great physicians of the continent. His investi

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Dr. Klotz is the youngest son of Dr. Otto Klotz, Dominion astronomer of this city, and it will probably interest Ottawans to learn that three of the latters sons are making their mark in the medical profession, the one who leaves Montreal to go to Pittsburg; Dr. Max. Klotz of this city; and Dr. Klotz of Lanark.

Dr. Oskar Klotz has created for himself a European and American reputation for original research work in many branches relative to the subject of Pathology and the Royal Victoria Hospital made every effort to retain his services. He spent five years in Europe studying this subject and has visited Germany twice. Dr. Oskar Klotz is an ex-student of the Ottawa Collegiate Institute, and when he matriculated from there attended Toronto University where he took his degree. The position which he is accepting carries with it the supervision of the pathological department in the Pittsburg college hospitals of which there are eight.

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1905

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In

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meinen lieben
Eltern

GEWIDMET

Den 31st. März.- 1906

From the 18th annual Report of the Assoc. of Executive Health Officers of Ontario. – Held at Peterboro. 1903

SERUMS IN THEIR RELATION TO DIPHTHERIA AND SCARLATINA.

By O. Klotz, M.D., Ottawa, House Physician, Contagious Diseases Hospital.

Gentlemen:—As the subject embraces the use of a serum in both diphtheria and scarlatina I shall deal with them separately. The serum used in the former we look upon as an antitoxin or neutralizing agent, in the latter it is still an open question just what relation exists between the serum used and the latent vital forces in the body.

The use of a diphtheritic antitoxin as a curative measure has been so fully covered in literature of recent years that it would be quite out of place for me to speak of it at length in that light. An analysis of the enormous amount of statistics collected shows us that the death rate has been reduced in diphtheria to about one-half since the introduction of antitoxin; and this improvement in the deathrate will yet increase with the earlier use of antitoxin and also in the improved methods in the production of the serum.

Instead of merely quoting statistics, however, I wish more fully to enter upon a discussion of the actual effects of the diphtheritic antitoxin on the human body and also the dosage.

At the Ottawa Isolation Hospital, from which my conclusions regarding antitoxin are drawn, all the cases of diphtheria occurring within the city are now being received. This institution gives one an unusually good opportunity to deduce certain conclusions, as cases of all degrees of severity are admitted. The statistics from former isolation hospitals have been somewhat prejudiced, as usually only malignant cases or patients *in extremis* were sent in.

With the present system of sending out culture media and swabs from the civic laboratory which is at the hospital to the physicians of the city, most of the cases have been previously diagnosed bacteriologically. This then leaves the cases only to be "quantitatively diagnosed"—or what dose of antitoxin does the patient require?

Up to five years ago antitoxin was mainly an experiment and the patient was given a varying dose and then left to nature, hoping that a sufficient quantity of antitoxin had been given to obtain favorable results; but now when antotixin is beyond its experimental stage the dosage must be summed up mainly from two points. First, from the extent of the pseudomembrane present, and, secondly, from the condition of the pulse.

The extent of the membrane gives us an idea as to the quantity of toxins which may be produced at the site and the condition of the pulse indicates the amount of toxaemia already existing. Nasal involvement of the diphtheritic process has a serious import as the extent of the membrane cannot be properly estimated, and it is found that the toxins are more readily absorbed from the nasal mucous membrane.

Too much reliance must not be placed on the general appearance of the patient. Too often do we find a patient striving to belittle a "sore throat," which, on examination, shows extensive patches of pseudo-membrane, and the presence of Klebs Loeffler bacilli; or the reverse is seen when the patient is suffering from some concomitant disease which magnifies the objective symptoms and would lead us to believe that the diphtheritic process is more severe than is actually the case.

In all uncomplicated cases of diphtheria the pulse rate is increased rapidly and is directly dependent on the amount of toxic products absorbed from the local site. A rapid pulse gradually or quickly slowing, after the acute symptoms have passed should be regarded as a grave symptom. In laryngeal diphtheria in which there is little if any membrane visible you must be guided wholly by the condition of the pulse and the amount of dyspnoea present. Though again the dyspnoea is often misleading, being out of all proportion to the amount of membrane present and the existing toxaemia. The dyspnoea is due, in a great part, to the oedema of the tissues for which these parts are so well adapted. Post-mortem examination has shown that a pseudo-membrane of small area situate in the region of the glottis may set up an extensive serous infiltration, sufficient to impede respiration.

The influence on the mortality of the stage of the disease at which the serum treatment is begun is very great. Reports from the Imperial Board of Health, 1895-96, covers a series of over 13,000 cases treated with antitoxin. The mortality of the cases treated on the first day was 6.6 per cent., second day 8.3 per cent., third day 12.9 per cent., fourth day 17 per cent., and fifth day 23.2 per cent.

In a moderate case of diphtheria the following dosage may be considered adaquate: To a child under one year 1,500 to 2,000 units. Doses of 500 units I should consider useless, and only a torture, as the dose must certainly be repeated in a short time. To a child over one year 2,000 to 3,000 units in all moderate attacks. From 4,000 to 5,000 units should be used for severe cases, and in all laryngeal cases of any age. These doses are to be repeated at the end of twelve hours if no improvement is evident or if there are no signs of the membrane loosening or "melting away." A third dose or even more may be given if necessary at the end of another twenty-four hours. Children under a year stand antitoxin remarkably well. Two of our cases, aged four and seven months, respectively, received 6,000 units in doses of 2,000 units at intervals of twenty-four hours.

At the present time when all the cases of diphtheria must be treated in the hospital I hold that it is a saving to the institution to use anitoxin in sufficient quantity. We derive most of our patients from the poorer classes, and the expense of their keeping falls on the treasury of the municipality; hence. every day longer in quarantine is a needless expense. I feel confident that through the liberal use of antitoxin we have at the Ottawa Civic Hospital materially lessened the quarantine stay of these patients. All our cases are discharged after two negative cultures have been made from the throat. There are stray cases, of course, in whom diphtheria bacilli linger for weeks, after the disappearance of the pseudo-membrane, and in whom there is no clinical evidence thereof, but who are a source of danger to others. There are exceptional cases to be found everywhere, and who cannot be freed from infection by the use of antitoxin. Including all these protracted quarantines our average stay in the hospital of undoubted cases of diphtheria has been 17.8 days. The average cost per capita per diem at a hospital is about 85 cents, and this is half again as much as 1,000 units of antitoxin costs. Do we need a more conclusive evidence for the more general and liberal use of antitoxin in the hospitals?

Of the ill effects of antitoxin on the system I have seen none more serious than a transient urticaria. This is, to say the least, very annoying to the patient, but never lasts more than twenty-four to forty-eight hours. I have noticed that in all cases in whom an urticarial rash appeared, it was usually a mild one or one which had received more antitoxin than was necessary to neutralize the quantity of toxin present in the body. Thus do we find also that this untoward symptom is prevalent among those receiving antitoxin as a prophylactic measure and who have no toxic product in the system to act as a neutralizing agent.

Of the cardiac complications due to the serum which the opponents to antitoxin have noted in statistics, I can give only one explanation, based on the result of 200 serum cases. Cardiac complications, ending either fatally or with serious impairment, result in those cases who had received an insufficient dose of antitoxin, or those who would have increased our mortality lists had they not been so treated. Degenerative changes, both in the nerves (the vagus being particularly attacked) and in the myocardium are caused by the toxins of diphtheria, and if these toxins are in such quantity or remain unneutralized by early and sufficient doses of antitoxin, we may expect to see the post-diphtheria cardiac complications still our chief source of danger.

Another point worth considering is that the number of intubated cases has fallen off remarkably. In the 200 cases our tracheotomy set has never been called into use and intubation was performed in only eight cases.

Sudden death occurs in diphtheria as we all know, anuria and convulsions have been frequently seen; heart troubles, functional and organic, are of frequent occurrence, but there is no evidence that any one of these is due to antitoxin—on the contrary, we know that many of these complications can be avoided by the early and sufficient administration of the serum.

At the Ottawa Civic Hospital we pin our entire faith on antitoxin as the curative measure for diphtheria. In children who resist local treatment, and in whom it would be a constant fight, exhausting to the patient, to spray their throats, we omit this part of the treatment entirely. Local antiseptics are to be looked on more as a prophylactic measure to keep the wards clean and to prevent reinfection of other throats.

Swabbing the throats with tincture of iron or iodine, as was the practice in pre-antitoxin days, should be considered obsolete and discontinued. These solutions tend to coagulate the necrotic tissue and thus form a leathery covering over the infected site, protecting the bacilli underneath and preventing subsequent antiseptics from having any effect. A very good example of this was shown in our Case No. 172. This patient, a boy aged thirteen, had been treated at home for six days. The treatment consisted in swabbing out the throat with strong tincture of iodine until the tissues had changed to a large slough. No antitoxin had been used. On admission to hospital the fauces, pharynx and uvula were covered with a dark brown membrane; the surface of the membrane gave a "negative" culture, while another culture, taken from the raw surface underneath, gave a "positive" result for diphtheria bacilli. In this case the local treatment was omitted for two days, in which time all the necrotic tissue pealed off. On the following day the abraided surface was covered with a thin, creamy pseudo-membrane, giving a pure culture of Klebs-Loeffler bacilli.

Next to antitoxin one must resort to a restorative and supporting treatment. Fluid diet, given frequently and in small quantity; stimulation with strychnia and alcohol being resorted to early.

The mortality in diphtheria at the Ottawa Civic Hospital, including deaths which occurred within a few hours after admission, has been 5.2 per cent.

In speaking of the use of antistreptococcic serum in scarlet fever, I shall be as brief as possible and deal more directly on the results of 49 cases treated in this way at our hospital. The literature up to the present time on the use of a serum in scarlatina is very meagre.

Before considering the serum treatment, however, let me first give a classification of the types of scarlatina which we must bear in mind, for all cases of scarlatina are not to be treated at random with the serum. The therapeutic value of antistreptococcic serum is to be found in only a certain class of scarlatinal patients, as I will attempt to show.

1. Simple Scarlatina. A benign disease in which the disturbances are caused by the toxin itself, but no one symptom predominating over the others. The case runs a very mild course with no complications. Serum treatment in these cases is uncalled for and will prove of no benefit. It will neither abort the course of the disease nor will it have any effect on the temperature. The only excuse for treating this class of patients with antistreptococcic serum would be as a prophylactic measure against a streptococcic invasion, but this is quite unnecessary in ordinary cases.

2. Malignant Toxic Scarlatina. This is the type which has been so prevalent during the last year in Ontario, and which, usually, if the case was not fatal, changed into the third type during the second week after the onset. In the pure malignant toxic type the patient suddenly becomes violently ill, stupor and coma set in from twelve to twenty-four hours, and death results in thirty-six to forty-eight hours after the onset. The patient is completely overcome with the scarlatinal toxaemia and seldom rallies. The temperature rapidly rises to 105 or 106 degrees, and remains unchanged by hydrotherapy or internal treatment. Death frequently ensues before the characteristic eruption is seen, and the diagnosis is made from the prevailing

epidemic or other cases of scarlatina in the same family. These cases resist all form of treatment, and the results obtained by the use of antistreptococcic serum are no more favorable. But of these the few cases that do not succumb usually fall into the class in which the use of antistreptococcic serum gives [redacted] commendable results. These latter form a very small percentage of the malignant type, and after recovering from the stupor enter upon a long and tedious convalescence which is interrupted by various complications arising from streptococcic infection. To combat this latter condition the serum is administered and with most happy results.

3. The third type includes those cases which we may call mixed infections from the beginning. These cases show all the clinical symptoms of a scarlatina simplex, together with those of a secondary infection. In them we see the early enlargement of the glandular system, the cervical and inguinal glands being particularly affected; there are indefinite pains in the joints—usually the knees and ankles; nervous symptoms are marked—twitching of face and limbs, and a trembling tongue ; prostration is usually marked. The streptococcus can be isolated from the mucous membrane of the pharynx and also from the blood. It is in this type that the antistreptococcic serum is of most benefit. We must bear in mind that the pharynx has an angry, red appearance which is more extensive than in scarlatina simplex. The serum is of value only in those cases whose lesions are due to the streptococcus.

During life the streptococcus may be isolated from the mucous membrane of the mouth, and from the blood and various organs of the body of a scarlet fever patient. Again, post-mortem examination shows the almost invariable presence of the streptococcus in the vital organs, the heart blood and the bone marrow. In 701 cases examined by Baginsky and Sommerfeld, they demonstrated the presence of streptococcus in 696 cases; from this they concluded that the streptococcus is an etiologic factor of scarlet fever.

The so-called complications and sequelae of scarlatina, consisting of otitis media, adenitis, abscess formation, gangrene, following suppuration of the tissues, albuminuria, rheumatism and so on, which prove of serious moment, not only in the individual case, but even for entire epidemics and practically shaping the complexion of the disease and its prognosis, are not regular attendants upon scarlatina, but so often form a part of the picture that a distinct relationship to it is unquestionable. Mild nephritis is, in all probability, usually due to the action of the scarlatinal toxin on the kidney, but often the inflammatory condition is caused by a direct streptococcic invasion.

Purulent discharges from scarlatinal patients show the constant presence of streptococci along with other germs. Regardless of the exact proportion of cases in which the streptococcus is found, it is shown that this germ is the most common mixed infection found in scarlatina, and its usual point of entrance is by the pharyngeal mucous membrane. On this important point we base the rationale of the therapeutic value of antistreptococcic serum. The condition is quite similar to that found in puerperal septicaemia, in which streptococcus can also be isolated from the blood and other organs. It would, perhaps, be well for us to speak of the former condition as scarlatinal septicaemia, for certainly, the streptococcus bears a closer relationship to scarlatina than mere chance.

On the subject of streptococcic infection there has been much experimental work done. At first this was carried out on animals, proving that a streptococcic septicaemia could be produced by the injection of the germ. Later, the experiments were carried on a step further, attempting to produce an immunizing serum against streptococcic infection in animals. Some very favorable reports have been given us in this line of work by Baginsky, Aronson, Sommerfeld and others. A serum was produced which would save the animal from death if administered within twenty-four hours after inoculation with a streptococcic culture. The antistreptococcic serum had the virtue to dispel the germs from the system, having a sort of bacteriolytic action. Hence Aronson argued that the antistreptococcic serum was indicated as long as any of the germs could be demonstrated in the blood.

Reasoning along these lines the antistreptococcic serum was eventually tried in secondary infections in scarlatina. The serum had proved successful in other cases of septicaemia of streptococcic origin, why should it not prove beneficial in scarlatinal septicaemina?

The action of the antistreptococcic serum differs from that of diphtheria antitoxin, in that its virtue lies not so much in neutralizing the toxins present in the system as in exerting a bacteriolytic reaction in the tissues. It has been quite conclusively shown that the serum in itself does not act as the direct germicide—as the serum forms a good culture medium for the streptococcus. Therefore, we must look to something else in the animal body, which stimulates cell activity, or which by an inter-action with the serum brings about a bacteriolysis.

Let me review some of the clinical histories of cases treated at the Ottawa Civic Hospital. We began using the serum in the first fifteen cases as a routine treatment, giving it to both mild and severe cases. This, we found, quite valueless in pure cases of scarlatina; though I might state that the mild cases so treated suffered no complications or sequelae, which we know often follow even the mildest attacks. We are not justified to draw any conclusions whether the serum had any prophylactic value, as the number of cases is too small.

Following this the serum was used only in those cases which showed such complications as are seen in secondary infections, or in whom we could isolate the streptococcus from the blood. In many cases which early in the disease showed oedema of the neck, enlarged and tender glands, indefinite articular pains, with much congested throat, marked prostration and stupor, we obtained the most favorable results. Everything in them pointed to a severe toxaemia, not alone from the causative agent of scarlatina but also from a streptococcic invasion.

The initial dose was usually 20 c.c., which was followed in twenty-four hours by a second dose of 10 c.c. Usually this was sufficient, but we did not hesitate to repeat a third or fourth injection of 10 c.c. each in more severe cases. The largest amount given was 90 c.c.

The first favorable sign after the administration of the serum is a fall of temperature, sometimes resembling a crisis. (This is well shown in the appended chart of Case No. 185.) The drop in temperature usually comes on from four to eight hours after the injection. Then follow in order the disappearance of the nervous symptoms, a clearer intellect, a reduction in the glandular enlargement, and after twelve hours the painful arthritis becomes less marked. Perhaps I can do no better than cite one or two of these cases.

Case No. 36, female, aet 8. Child of a poor family, has always been well, though never robust. Became suddenly ill on January 16th, with vomiting, some headache and sore throat. Child remained in bed all day of 17th. Scarlatiniform rash appeared on chest on evening of 17th. Child admitted to hospital on 18th. On admission found child poorly nourished, rash beginning to fade on body but still distinct on limbs, in axillae and about pubes. Marked redness of pharyngeal mucous membrane, fauces and palate, moderate swelling of tonsils and tongue. Tongue coated with papillae showing through. No pseudo-membrane visible on throat. Patient stupid and talking incessantly, but answers questions intelligently. Delirious greater part of night and lies with eyes partly closed, difficult to rouse child; while resting there is a continual twitching of the limbs. Glands of neck hard and enlarged; loose tissues of neck, oedematous and swollen. Inguinal glands also enlarged. Temperature 103. Pulse 130.

January 19th. Child about the same condition as yesterday. Patient shows marked stupor, responding to questions either not at all or with an unintelligible answer. Does not seem to notice what is going on about her. Pupils dilated. Temperature 105. Pulse 156. At 11 p.m. 10 c.c. antistreptococcic serum given.

January 20th. Patient rational for short time during morning. In afternoon was unconscious again. General condition about the same as on previous day. Neck still swollen on both sides under angle of jaws. Still complaining of sore throat. Throat culture negative for Klebs-Loeffler bacilli, but contains streptococci. Face, thin and pinched. Twitching of arms and legs marked. Eyes dull. Tongue coated a dirty brown. Albumen present in urine. Complained of pains in limbs and back. Sleeps very little, tosses about and tries to get out of bed. At 10 p.m. 10 c.c. antistreptococcic serum was given. Patient somewhat quieter during the night, but very weak and drinks a great deal of water.

January 21st. General condition about the same. Still delirious and restless. Pulse weak and irregular during afternoon. Respirations, shallow and panting. Glands on side of neck still hard and painful. Temperature 104. Pulse 107. Respiration 30. At 10 p.m. 10 c.c. antistreptococcic serum given

January 22nd. Vomited several times during the day; otherwise condition about the same. Patient very noisy at times; complains of very sore throat and cannot swallow. Temperature 104. Pulse 146. Respiration 36; 20 c.c. antistreptococcic serum administered at 11 p.m.

January 23rd. Temperature dropped this morning to 100. Patient much quieter, delirious only a short time; takes nourishment better. Condition of neck improved with oedema subsiding. Pains in limbs not so severe. General condition of patient improved.

The change in the appearance of the child on the 24th was indeed remarkable. From the half-conscious, drowsy and sullen condition of the child there was a change to a quite rational one.

No further serum was given. Patient improved considerably during the following week. Enlarged glands diminished and oedematous condition of neck disappeared. The albumen was absent from the urine at the end of second week. The patient became much brighter and the mind was clearer. Pains in back and limbs left entirely, and at the end of the third week the patient was convalescing favorably.

After each administration of serum there was a drop in the temperature. At first it was slight, but later became quite distinct and resembled a crisis. In this case I consider the initial dosage was too small, and would have expected still better results from a larger amount of serum given. During the convalescence patient suffered no concomitant rheumatism, otitis media or other complications. Patient gained about seven pounds in weight and was discharged in good condition at the end of nine weeks. No change was noted in the rate of desquamation.

Case No. 169. A sister of the previous case. Was admitted shortly afterwards with almost an identical history. The form of scarlatina was the same in both, each showing extensive glandular enlargements and severe nervous symptoms, but the throat culture in the latter gave the presence of Klebs-Loeffler bacilli, along with streptococci and staphylococci. For the first three days the temperature remained between 105 and 106 degrees. On the fourth day there was a drop to 102 degrees, and from then on the temperature gradually came down to normal at the end of three weeks.

In this patient the initial dose on the first day was 20 c.c. of the serum and was followed by 20 c.c. on each successive day for two days, with a final dose of 20 c.c. on the eighth day. Besides the 80 c.c. antistreptococcic serum this patient also received 4,000 units antitoxin.

As in the previous case the delirium ceased at the end of five days; there was a constant reduction of temperature following the administration of serum, and there were no complications following, during convalescence.

To sum up briefly the results in the forty-nine cases treated with serum have been:

There were two deaths.

Five cases developed albumenuria.

There were only four who suffered from otitis media.

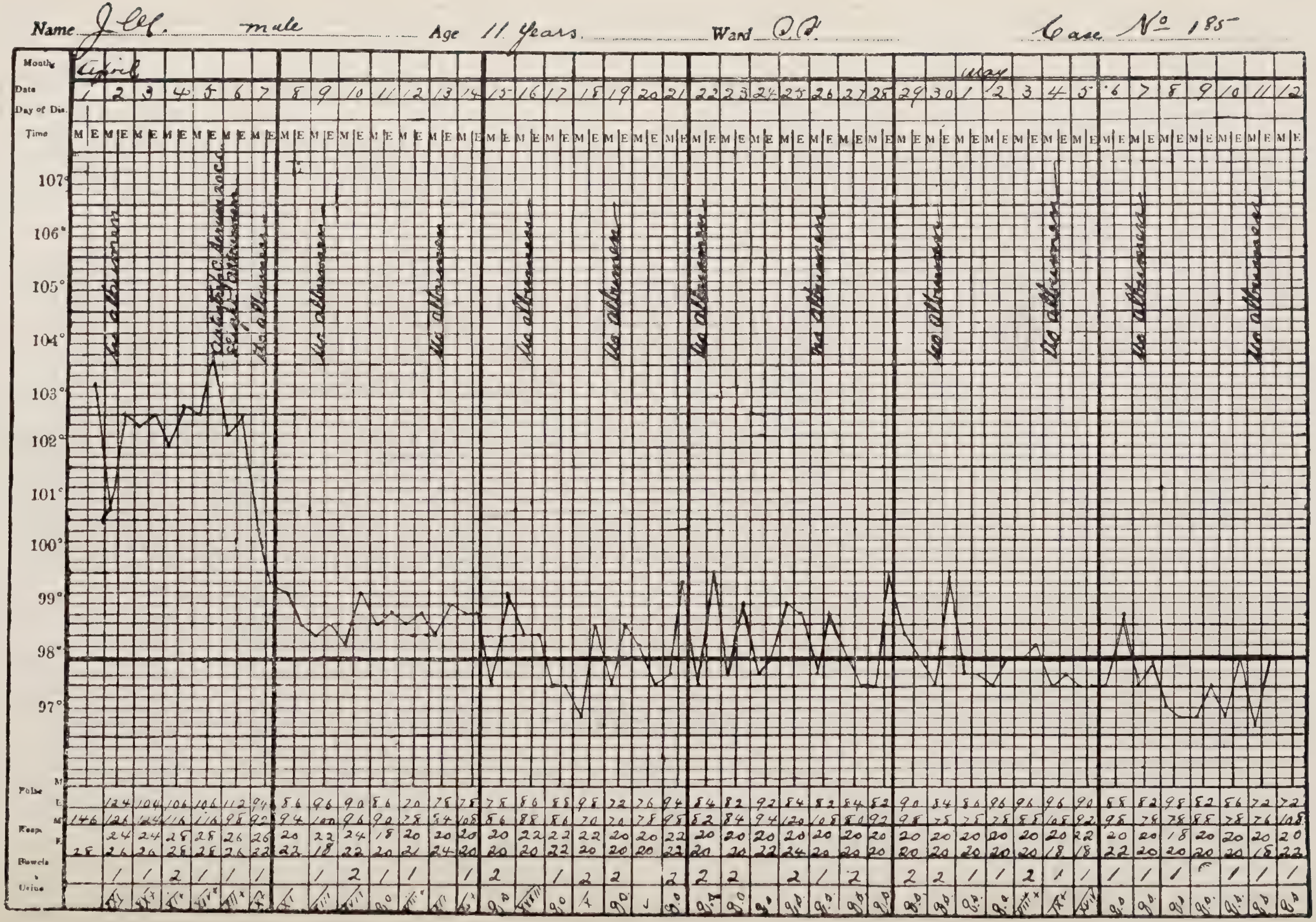
Two cases suffered from arthritis.

One with functional cardiac complications.

Death ensued in the one case on the sixth day, the temperature during the whole illness ranging from 103 to 106 degrees. This case was, to my mind, one of the malignant toxic scarlatinas. The other case died after a protracted illness, being admitted to the hospital suffering from scarlatina, diphtheria and varicella.

A point I wish to emphasize in regard to the antistreptococcic serum, as well as in diphtheritic antitoxin, is that the serums must be administered early if we are to expect any benefit from them. Where an adenitis has already proceeded to suppuration it is false to imagine that the injection of the serum will dispel this, and you will likely be disappointed in your expensive treatment.

It is still a question of experiment and investigation to discover the materies morbi of scarlet fever, but having once found this, we may hope to be on a fair road to the production of a true scarlatinal antitoxin.


Name J. Cl. male Age 11 Years Ward O.P. Case No 185
Month
Date
Day of Dis.
Time
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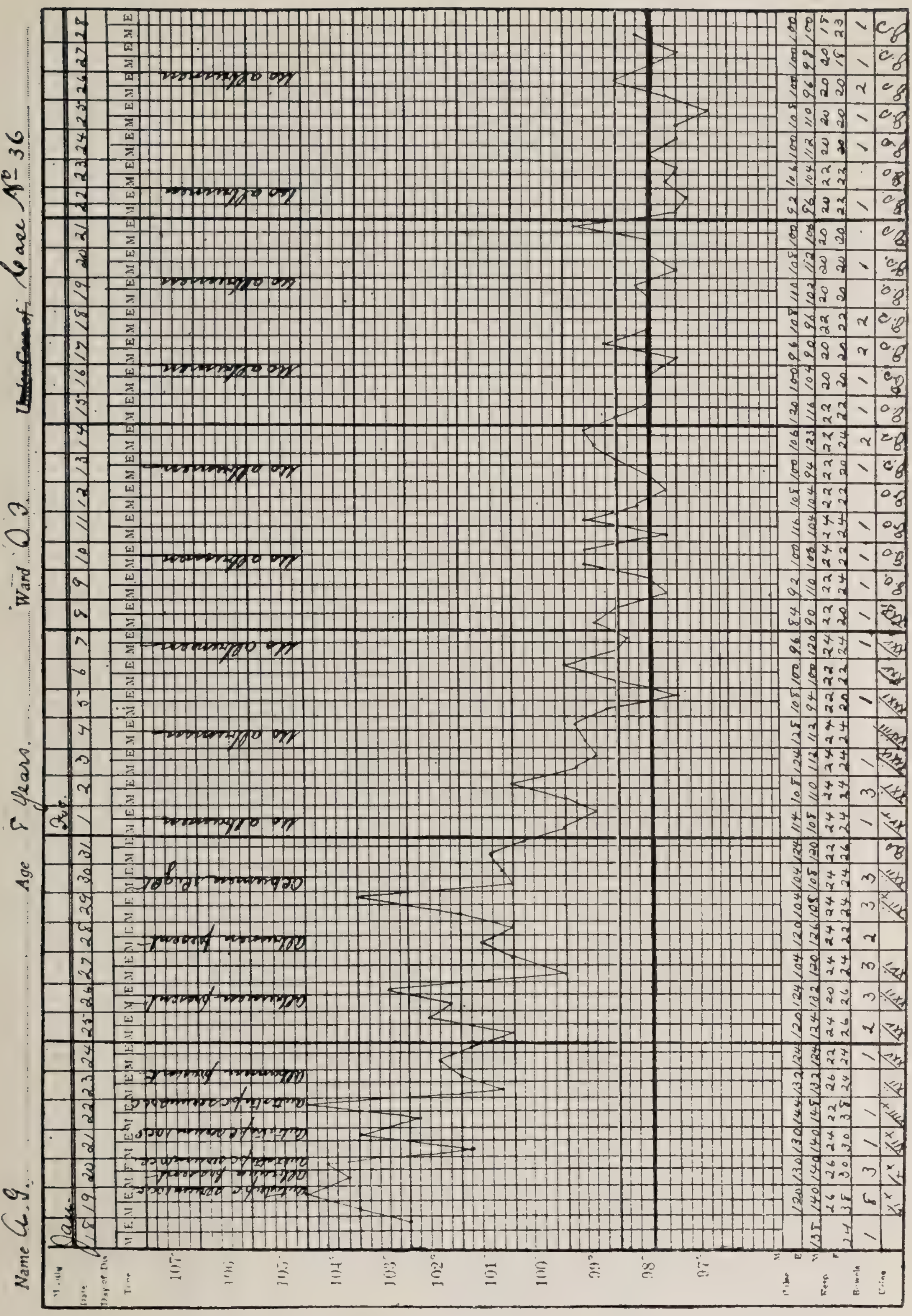
May
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
Pulse
Resp.
Bowels
Urine
No albumen


Name A. G.
Age 5 Years.
Ward O. ?
Case No 36
Date
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107°
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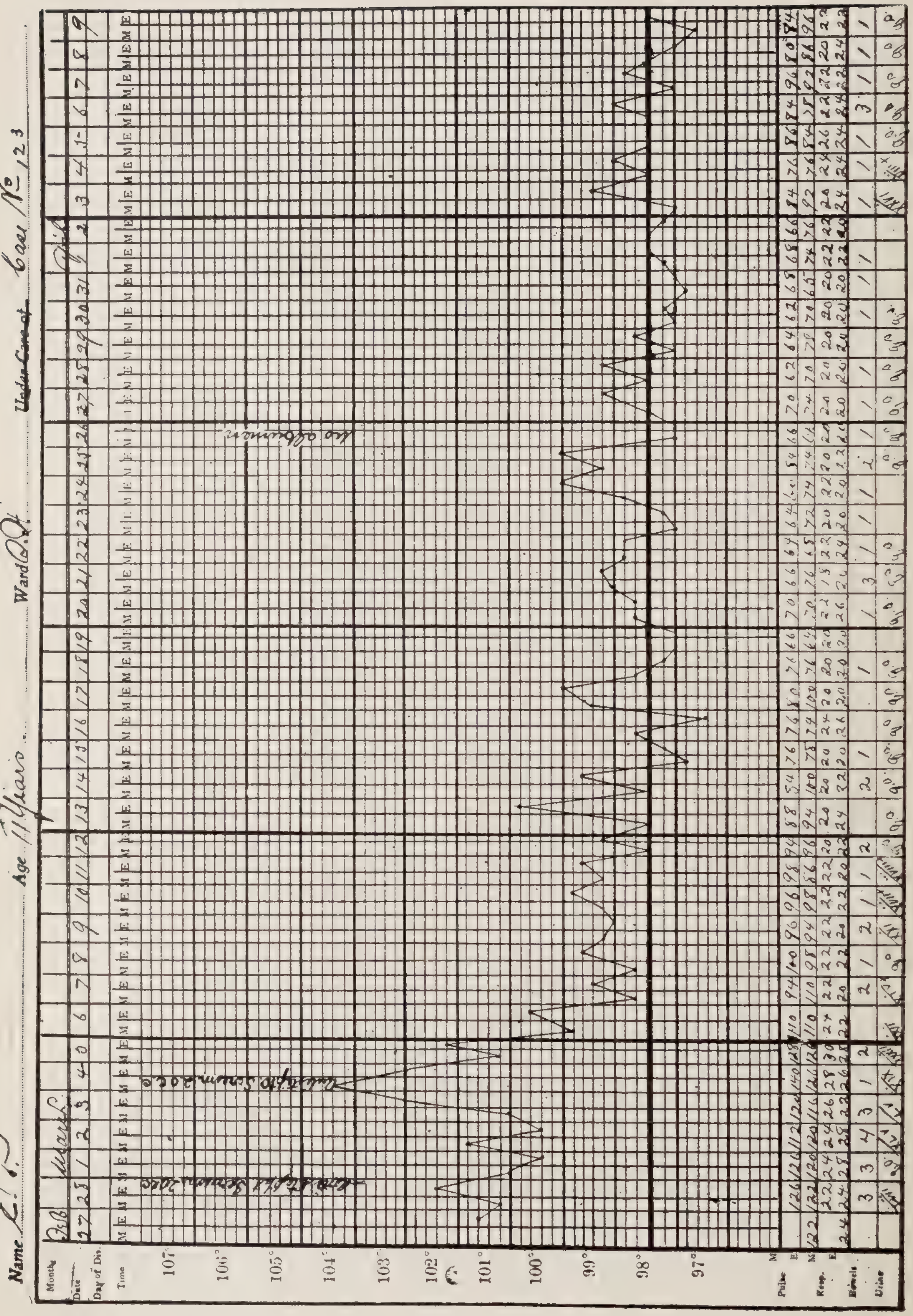
Name E. P.
Age 11 years
Ward
Under Care of
Case No. 123
Month
Feb.
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Day of Dis.
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M E
107°
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97°
Serum 20cc
Serum 20cc
No albumin
Pulse
Resp.
Bowels
Urine

ON THE LEUCOCYTOSIS IN SCARLET FEVER

OSKAR KLOTZ
(Governor's Fellow in Pathology, McGill University, Montreal.)

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CHICAGO

ON THE LEUCOCYTOSIS IN SCARLET FEVER.*

OSKAR KLOTZ,
Governor's Fellow in Pathology, McGill University, Montreal.

IN the following observations an attempt has been made to verify the observations of previous writers, and further to note the amount of leucocytosis in different stages of scarlet fever, in different grades of severity of the disease, and also the effect of the various complications and sequelæ on the white blood count. Though there are changes in the various elements of the blood in scarlet fever, erythrocytes as well as leucocytes, it is to the condition of the white cells that I shall confine myself.

Pick, Halla, Rieder, and Kotschetkoff were among the first to report their observations in blood analysis of scarlet fever patients, and all were agreed on the constant presence of a leucocytosis. Their actual figures as to the intensity of the leucocytosis vary considerably, due no doubt to the varying intensity of the disease.

Kotschetkoff found, after examining the blood of twenty scarlatina patients, that the leucocytosis was directly proportionate to the severity of the cases, the variation lying between a minimum of 10,000 and a maximum of 40,000. The highest leucocytosis (40,000) he obtained in rapidly fatal cases. He found an early leucocytosis in all, reaching its height on the second or third day, then gradually decreasing to normal after six weeks. From his observations he concluded that neither temperature, lymphadenitis, otitis, nor nephritis had any effect on the number of leucocytes. In his cases the *überreiffen Formen*—the neutrophiles and eosinophiles—were increased in number to 85–98 per cent., but those having 95 per cent. or more of neutrophiles and eosinophiles ended fatally. These cells were found in greatest number on the second day of the rash, from which time on there was a daily decline to normal. After returning to normal, further complications had no influence on the count. In fatal cases the eosinophiles and neutrophiles remained at their maximum. Kotschetkoff found that the

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* Received for publication January 26, 1904.

eosinophiles were increased, except in fatal cases, from the third day on till the third week, to as much as 15 per cent.

Zappert, from his studies on eosinophiles, concludes that in fevers, during the febrile intoxication, there is a fall in the eosinophiles. After the disappearance of the fever, the eosinophiles gradually ascend to normal. In scarlatina he agrees with Kotschetkoff in the early diminution of the eosinophiles, with a subsequent rise, reaching the maximum at the end of the third week.

Of the more recent writers on the subject, Sacquépée found that, in older patients, twenty to twenty-two years of age, the eosinophiles were increased early in the disease (fourth or fifth day), and the increase persisted for three or four weeks. The apparent increase in the mononuclears, he holds, is due to a more rapid decrease in number on the part of the polynuclears. Cervical adenitis and parotiditis, as complications of scarlatina, both show more or less leucocytosis. In fatal cases of scarlatina he observed a hyperleucocytosis with "abnormal" forms, which he found in none but malignant cases. The polynuclear cells, showing a decided acidophile nature, he regarded as degenerated eosinophiles.

Reckzeh found a maximum leucocytosis of 41,000, on the fourth to the ninth day. The decline in the number of leucocytes, he states, is gradual, and reaches its normal toward the third week. He found that adenitis, joint affections, and endocarditis each causes an increased leucocytosis. On the contrary, nephritis is followed by a decrease in the number of white cells. The lymphocytes are fewer at the onset, but later increase as the polynuclears decrease. The eosinophiles in his series varied from 1 to 12.5 per cent., reaching the maximum about the eighth day. He believes that the unknown scarlatinal toxin stimulates the blood-producing organs (as regards the leucocytes) to a greater activity, with a consequent increased white cell count.

Reckzeh gives a very full tabulated statement of the successive blood counts throughout the course of each of his ten cases, often as many as a dozen, and extending from the second or third day of the fever to the end of the fifth or sixth week. What appears to me not a little remarkable is that, in the majority of these counts, he reports either a low count or a total absence of eosinophiles. This he does not refer to in his summing up, but, on the contrary, lays stress upon the eosinophilia. Judging from my observations, his "0" in many cases means either that he has failed to count the eosinophiles, or else that he has regarded the faintly stained, coarsely granular oxyphile cells among the neutrophiles. Reference to the plate accompanying this paper will, I think, establish that these are truly to be classed as eosinophiles proper.

Rille* observed that in two moderate cases of scarlatina the eosinophiles were moderate in number, though there was a leucocytosis varying from 8,800 to 18,200. In a fatal case he found the eosinophiles increased 5.3 to 7.77, with a leucocytosis of from 15,000 to 20,000.

Plantenga, comparing the results of his examinations of measles and *Rötheln* with those of scarlatina, concluded that a diagnostic point could be made of an early increase in the polynuclears in the two first-mentioned diseases. Reference to my tables does not support this view.

Mackie found in examining the blood of scarlet fever patients that the leucocytosis was marked in mild or moderate cases, while in unfavorable cases the leucocytosis was less marked or even absent.

While writing this article, after the completion of my observations, my attention was drawn by Dr. Hektoen to a singularly full and able paper on the subject by J. M. Bowie. Bowie examined many more cases than are taken up here, but I was very much struck with the similarity of his observations with those here given. What would appear to be discrepancies in our results I think can be explained by two facts: one, the constant variation in the intensity and nature of scarlatinal epidemics, and the other, our different classification of the varieties of the disease. No one will deny the great variation in the intensity and sequelæ to which scarlatinal epidemics are subject, which necessarily must also affect the leucocytosis. Bowie divides his cases into scarlatina simplex, scarlatina anginosa, and fatal cases. In the first group he considers only the mild, I presume uncomplicated, cases. In the second group he places all moderately severe cases, with and without complications. In my series, those spoken of as moderately severe I divide into two classes—the pure uncomplicated cases, and those with complications of adenitis, otitis, joint affections, and so on. Bowie also divides his fatal cases into two groups—"those that never recover from the primary shock of the fever, and those that make a bold bid for life, but die eventually from exhaustion and toxemia." Two of the cases to be considered later come under the first heading; a third, under the second. I shall take up his paper more fully in the summary, and compare the results with the present observations.

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* Cited by Reckzeh.

Remembering the frequent discovery of streptococcal complications in this disease, it becomes worth while to note the observations made regarding the variations in the leucocytes in septic, and more particularly in streptococcal, disorders.

Cabot has tabulated a series of cases of septicemia, and found that forty-two out of fifty-six gave a leucocytosis varying from 9,000 to 77,500. The leucocytosis was of the polymorphonuclear type, with a disappearance of the eosinophiles and a diminution of the lymphocytes. He further reports the examination of three cases in which the streptococcus was isolated, and showing a varying leucocytosis of from 24,800 to 46,000.

Rieder notes in a fatal case of sepsis that the eosinophiles are wholly absent.

Residence in an infectious disease hospital (the Ottawa Isolation Hospital) gave me an opportunity of examining a series of fourteen cases of undoubted scarlatina. Only those cases were utilized in which the clinical history and symptoms indicated the unquestionable nature of the disease. As all the cases are from hospital practice, examinations were not obtained earlier than twelve hours after the onset; hence no results can be given as to the leucocytosis appearing during the incubation period.

The ages of the patients ranged from two and a half to fourteen years. The examination consisted in the estimation of the white cells per cubic millimeter, and the preparation of stained slides for the estimation of the relative count, and the minute detail of the cells.

The blood was obtained in the usual way from the lobe of the ear. The examinations were made late in the morning, usually just before noon, to obtain counts as nearly as possible under similar conditions, and eliminate the leucocytosis following digestion. The examination was made with a Thoma-Zeiss hemocytometer, using a 3 per cent. acetic acid solution, tinged with gentian violet, as a diluting medium. Three successive counts were made of the complete 400 squares each, making a total of 1,200 squares.

The films were made on coverslips and fixed in a mixture of equal parts of alcohol and ether, being stained later by the Nocht-Romanowsky method. For the determination of the relative numbers of white cells present at least 800 cells were counted. In studying the more minute structure of the cells a one-twelfth oil immersion lens and No. 5 ocular were used (Reichert).

As normally there is a considerably higher count of white cells in children than in adults, a leucocytosis was considered only in the presence of 10,000 or over per cubic millimeter. The normal adult differential count may be considered as,

	Per cent.
Lymphocytes	22–25
Polymorphonuclear neutrophiles	62–70
Eosinophiles	0.5–4

In infancy and childhood the percentage of lymphocytes is higher (40–60 per cent.), and the polymorphonuclears are only 28–40 per cent. (Cabot).

The cases examined in these observations were: two cases of very mild scarlatina, nine of moderate severity, and three of malignant scarlatina, two of which died within three days after the onset. Among the cases of moderate severity were also those developing adenitis, otitis media, nephritis, and arthritis. In two patients, counts were obtained before and after the administration of antistreptococcic serum.

MILD SCARLATINA.

The diagnosis in these two cases rested mainly on the mode of onset, the presence of other cases of scarlet fever in the same family, and the subsequent desquamation.

In the very mild type of scarlet fever the white cells showed little or no change in number or relative count. This, then, resembles the condition found in pneumonia, where we also meet with a normal, or even subnormal, count during a mild attack. The highest count obtained in these two cases was 12,780 on the second day, while the lowest was 7,400. The differential counts were normal. The conditions here also bear a close resemblance to those seen in measles, where the count is always low.

Case I, female, *æt.* 6—	2d day	12,780
	9th day	9,228
	11th day	10,740
	28th day	11,680
Case II, female, *æt.* 2—	2d day	9,440
	12th day	7,400

In each of these cases the rash consisted of a diffuse blush over the body, which disappeared by the fourth day; the sore throat was very slight; and desquamation was of the fine branny character. In neither case were there any complications during convalescence.

SCARLATINA OF MODERATE SEVERITY.

This type of the disease presents to us the most interesting series, for in it we meet with some running an uncomplicated course of scarlet fever, while others develop adenitis, otitis media, and so on, thus giving us several classifications to be considered.

During the first week we may consider all the cases of this type of the disease under the same heading, as up to this time no complications have arisen.

Viewing the leucocytosis as it occurs on the different days, the results are as follows:

Day of Disease	No. of Cases Examined	Average Count
2d	1	18,000
3d	5	32,260
4th	3	**33,185**
5th	2	29,505
6th	1	30,670
8th	2	23,060

The highest individual count obtained in the above series was 78,133 on the third day of the disease. No reason could be assigned for this high count, as the leucocytes numbered only

28,730 on the fifth day, while the case ran its further course of moderate severity with no complications. The lowest count during the first week was 11,370.

In the above series, it will be seen, the leucocytosis ascends till the fourth day—which just about represents the course of the intensity of the scarlatinal rash.

None of the patients had a temperature above 103.2°, and the highest temperature was usually on the second or third day. The curve of the leucocytes does not follow the temperature curve. While we may note a sudden drop in the fever, this does not denote a consequent fall in the number of leucocytes, which gradually decrease in number till the fourth or fifth week.

From the eighth day on, the cases must be divided under several headings as complications arise:

Pure uncomplicated cases.—Of the uncomplicated cases of moderate scarlatina in the series there were five, in which the counts resulted as follows:

Day of Disease	No. of Cases Examined	Average Count
15th	3	15,585
21st	3	13,260
25th	1	15,270
29th	2	12,890
34th	1	10,900

The tabulation shows a gradual decrease in the leucocytosis. During this time (from the end of the second week on) the temperature runs a normal course and desquamation is advancing. Desquamation of various intensity showed no change in the white-cell count.

Complicated cases.—One case, complicated with *adenitis* and *otitis media* (suppurative) during the third week, gave an increase in the leucocytosis. The discharge from the ear contained streptococci, staphylococci, and some large bacilli which were not determined.

Day of Disease	Count
15th	14,720
18th	Onset of adenitis and otitis media
19th	**18,460**
25th	16,250
32d	13,630

Two cases, complicated with *varicella*, gave an increased leucocytosis at the onset of the second disease (the rise in temperature being the first symptom) and before the appearance of the vesicles. The first case occurred on the sixteenth day, with a consequent leucocytosis of 20,470—a rise of nearly 7,000 over an examination three days previously. The second case developed varicella nineteen days after the onset of scarlatina, and showed a leucocytosis of 18,060 on the day previous to the appearance of the vesicles. Forty-eight hours after the appearance of the vesicles, when these were becoming opalescent, while others were dying, the white-cell count amounted to 16,632.

One patient developed a severe *arthritis* on the forty-fourth day after the onset of scarlatina. The ankles, wrists, and knees were particularly affected, while the temperature rapidly rose to 104 and 105°. The leucocyte count up to this time had been that of moderate severity, the highest count being 26,720. The count on the thirtieth day was 14,056; on the forty-fifth day, the day following the onset of the arthritis, this was increased to 32,740. The temperature remained persistently high for some weeks, returning to normal at the end of the ninth week. On the fifty-sixth day the leucocyte count was 18,900, and at the end of the ninth week this fell to 14,348. The patient left the hospital at the end of the tenth week, and although the temperature remained about normal, the arthritis persisted for some time.

MODERATELY SEVERE CASES—DIFFERENTIAL COUNT.

Now, considering the differential count in cases of moderate severity, the following results were obtained:

Period of Disease	No. of Cases	Total No. of Leucocytes Averaged	Maximum No. in Any Case	Minimum No. in Any Case	Average Percentage of		
					Polymorphonuclears	Lymphocytes	Eosinophiles
2d–4th day.........	5	25,130	78,133	11,370	89.1	9.8	1.1
4th–10th day.......	5	29,080	48,270	16,400	85.6	10.7	3.7
10th–16th day......	4	17,100	22,500	14,940	79.6	13.6	6.8
16th–22d day.......	4	13,080	23,060	9,300	69.2	14.5	16.3
22d–31st day.......	4	14,230	18,240	9,870	58.5	30.9	10.6
35th–42d day.......	3	11,400	13,500	8,900	59.3	47	0.5–4
Normal...............		9,500–10,500			30–50	40–60	0.5–4

The highest eosinophile count during the first week was 4.9 per cent. and the lowest 0.41. The highest polymorphonuclear count during the first week was obtained on the second day of the disease, the count reaching 96.7 per cent. of all cells. The lymphocytes were reduced in this case to 2.89 per cent.

In the early stages of the disease the polymorphonuclear cells show very poor staining qualities, the nuclei being indistinctly outlined. The chromatin films are much broken up and stain unequally. The fine granules outside the nucleus present a hazy purple appearance.

During the second week the highest individual eosinophile count was obtained, on the thirteenth day, there being 19.3 per cent. of these blood elements present. The coarse oxyphile cells stain very intensely; the granules are larger and are packed closely in the cells. The cells seem overburdened with the acidophile granules, and are readily broken in making blood films, in which case the field about the burst corpuscle shows many of these granules extra-cellular and well stained.

Toward the end of the second week the polymorphonuclear leucocytes also change in character. The fine oxyphile granules are now somewhat coarser and take the eosin stain more actively. These cells show their oxyphile character in all grades, from the faintly staining pink cells, whose individual granules can be distinguished, to those that show such intense and coarse staining that they closely resemble the true eosinophile leucocytes. This acid-staining characteristic was noted particularly in the severer cases which had passed the crisis.

From the end of the second week there was a constant decline in the proportion of the polymorphonuclears, while the lymphocytes increased. A lymphocytosis during convalescence was found only in a few instances, the highest proportion being 52.3 per cent. on the thirty-first day of the disease.

The eosinophiles run a course quite different from that of the other leucocytes. During the first few days the count is low, and even at times from day to day decreases, up to the fifth or sixth day, their number from this time on rapidly increasing, till a maximum is reached (in my series of cases) between the fourteenth and twenty-first days. This initial decline with a secondary rise fairly accurately followed, inversely, the temperature curve, which in turn denotes the existing toxemia. Hence it would seem that during the time of the extensive elaboration of scarlatinal toxins in the body there is a low eosinophile count. From the end of the third week, in nearly all cases, the eosinophiles gradually decrease during convalescence. Slight exacerbations of temperature had no effect on the proportion of the coarse oxyphiles.

The cases complicated with adenitis and otitis media showed an increase mainly in the polymorphonuclears. In acute nephritis the relative count was not constant; the polymorphonuclears were at first slightly increased, while later the lymphocytes rose in proportion to the total number of cells. The eosinophiles were never increased.

MALIGNANT SCARLET FEVER.

In this classification are considered two cases in which the toxic symptoms completely overshadowed all others, the patients becoming comatose within eighteen hours after the onset; and a third in which there was a persistent high temperature until death, at the end of three weeks. In each of the former cases but one count was obtained, death ensuing on the first and third day respectively. In one of these cases the examination was obtained fourteen hours after the onset, and the count amounted to 6,520. The relative count showed a complete absence of eosinophiles, while the polymorphonuclears were present in the proportion of 68.5, and the lymphocytes of 31.45, per cent. The second patient gave a count of 10,832 leucocytes in the proportion of—

	Per cent.
Polymorphonuclears	76.0
Lymphocytes	24.7
Eosinophiles	0.3

The interesting feature in both of the above cases is the small number of leucocytes present, many writers claiming a leucocytosis of 40,000 in malignant scarlatina. The loss in the eosinophiles follows the rule as seen in scarlatina of moderate intensity, in that their numbers are decreased in proportion to the existing toxemia.

The third example of this type of scarlatina suffered with a concomitant diphtheria, which bars any conclusions, as regards scarlet fever, to be drawn from it. At any rate, it is interesting to note that here, too, the leucocyte count was low, ranging (in four counts taken at intervals during three weeks) from 8,340 to 13,860. Also in the relative count the proportion of eosinophiles was reduced, numbering from 0.16 to 0.24 per cent. of the total white cells.

The polymorphonuclear cells stained poorly and did not show the acidophile tendency as seen in the preceding type. The nuclei showed fragmentation—an indistinct outline—and lay close to the periphery of the cell. The nuclei stained a pale blue, and the meshes of the intranuclear arrangement were loose and in many places transparent. The oxyphile granules in the eosinophiles had a washed appearance, as if these granules had partially dissolved in the cell substance which then took the acid stain.

CASES TREATED WITH ANTISTREPTOCOCCIC SERUM.

Two patients who toward the end of the first week of the disease received 30 c.c. antistreptococcic serum were examined. In each case a count was made immediately before the administration, and then a second count four hours after.

The results obtained were as follows:

	Case I		Case II	
	Before Administration	Four Hours After	Before Administration	Four Hours After
Total count	17,160	15,520	20,656	16,410
Relative count—				
Polymorphonuclears	82.1%	78.3%	88.4%	80.1%
Lymphocytes	16.8	18.3	8.7	12.3
Eosinophiles	1.1	3.4	3.9	7.6

The changes occurring were thus a diminution in the total number of leucocytes and a relative increase in the eosinophiles and lymphocytes. The increase was particularly noticeable in the eosinophiles, which at the same time appeared smaller than normal. No change was noted in the staining qualities of the different leucocytes. The diminution in the total number of leucocytes, it should be noted, is similar to that seen after the administration of antitoxin in diphtheria.

GENERAL SUMMARY.

Bowie, considering his *mild* cases under scarlatina simplex, obtained as high a count as 34,900. His average, however, in this class varied from 10,000 to 18,850. As a general rule, he states, the slighter cases have a low leucocytosis. In my own series the changes in the white cell elements, in mild scarlatina, are slight. The white cell count rarely reaches 20,000, and falls back to normal in the third, or even the second, week. A leucocytosis of any intensity is not the rule, nor are there marked changes in the proportions of the different leucocytes. As already suggested, the partial want of harmony between the two series would appear to be due to difference in the grade of case dealt with by Bowie and myself under this heading.

~~GENERAL SUMMARY.~~

As regards scarlatina of *moderate* severity, the series here recorded shows a constant leucocytosis of high degree—as a rule from 20,000 to 40,000; it may rise as high as 78,000. In this form the leucocytosis is more marked than that seen in either the mild or fatal cases. The height of the leucocytosis is attained on the third or fourth day after the onset of the disease, from which time it gradually falls to normal at the end of the fifth week. The duration of the leucocytosis varies with the severity of the attack, some cases falling to normal in four weeks, others being protracted into the seventh week.

The early changes are the rapid increase in the polynuclears, rising to as much as 93 per cent. of all the cells. Coincident with the increase of the polynuclears there is a fall in the number of lymphocytes. In uncomplicated cases the polynuclears have attained their maximum in the first week, and then gradually decrease, reaching their normal from the third to the fourth week or later according to the severity of the attack. Early in the first week of the disease the eosinophiles are found below the maximal limits of the normal count, and at times below the minimal limit. Then there is a rapid increase, which, during the second and third week, may attain a proportion of 19 per cent. of the total leucocytes. The cases showing a good resistance to the disease, and in which the prognosis is good, attain the maximum eosinophile count within the first ten days. The normal is again reached toward the end of the fifth week.

These cases of moderate severity appear to represent in part Bowie's series of cases of scarlatina anginosa. In these anginose cases Bowie found that the polynuclears were increased relatively and absolutely at first, attaining as much as 90 per cent. in one case. His highest counts were obtained on the third and fourth days, and in cases where there were no complications normal was again reached at the end of the third week. The eosinophiles, he noted, were decreased during the first few days. This decrease was evident for a greater length of time in the more severe cases, and an increase was evident only when the tissues were obtaining the upper hand of the toxemia.

In my cases I found that the eosinophiles varied according to the severity, not only in number, but also in appearance. In moderately severe cases, where the tissues were gradually overcoming the attack of the toxins, the oxyphile granules were well marked and crystalline in appearance. If the percentage of the eosinophiles is high, the individual cells are smaller than normal; the nucleus is more compact and stains a deep blue. The polymorphonuclear cells show a strong activity for acid stains. The oxyphile characteristic of these cells varies directly with the intensity of the disease along with the resistance exhibited by the body, or conversely. In severe attacks of scarlatina the prognosis is more favorable in those showing a marked oxyphile nature of the polymorphonuclear leucocytes, along with a high eosinophile count.

This matter of degenerative appearances seen in leucocytes at one or other stage of an infective process deserves attention. Until recently but little mention has been made regarding it in the literature. Arneth's paper in the *Deutsche medicinische Wochenschrift*, Nos. 1 and 2, 1904, is the beginning of a fuller study of the subject. For the present, in the absence of wider knowledge, it seems to me advisable merely to call attention to the fact that degenerative changes of the nature described are to be met with in the leucocytes of scarlet fever, without attempting to discuss their ultimate nature. The accompanying plate gives, as nearly as I can express it, the varieties in the appearance of the oxyphile granules.

Complications and sequelæ of lymphadenitis, arthritis, varicella, and otitis each show an increase of 4,000 to 12,000 in the number of white cells, the polynuclears showing the greatest change. Nephritis may show either an increase or a decrease in the leucocytosis, depending on the stage and severity of the disease it accompanies. Weakly patients who have had a hard struggle with the original scarlatinal intoxication, and whose resistance has not been brought up to a point to throw off a new attack on the kidneys, show a fall in the number of white cells. On the contrary, patients who during convalescence have a transient, more or less severe, attack of nephritis have an increased leucocytosis.

Reverting again to Bowie, he found that all his cases of nephritis showed an increased leucocytosis, but he notes that the cases of short duration and more favorable prognosis have the highest leucocyte count.

Malignant scarlatina — in which I do not include severe complicated cases, but only those which are intensely affected by the scarlatinal toxin alone — tend to show a low total leucocytosis, and also a diminished eosinophile proportion. Compared with the previous type of the disease, it is thus seen that the leucocytosis varies according to the severity of the disease up to a certain limit, when the extreme toxemia either paralyzes the cell-producing organs, or else causes a cytolytic action. At any rate, there is a diminished number of white cells.

In these malignant cases the polymorphonuclear cells do not show the oxyphile character seen in the cases with a favorable prognosis. The indistinct appearance of the oxyphile granules, with the "washed," pink-stained protoplasm of the few eosinophiles present, would tend to give one the impression that there is cytolysis of this type of cell going on in malignant scarlatina. We no longer find the eosinophiles with the protoplasm densely packed with oxyphile granules, and a deeply stained nucleus.

Bowie lays stress on the low total leucocytosis and the almost total absence of the eosinophiles in unfavorable cases, but he makes no note as to the changes in the staining qualities of the different kinds of cells.

A further study determining the part played by the oxyphile granules is required to clear up the significance of the eosinophilia.

In conclusion, I wish to express my thanks to Dr. Hektoen for valuable references to articles which otherwise would have escaped me, and to Dr. Adami for suggestions and counsel.

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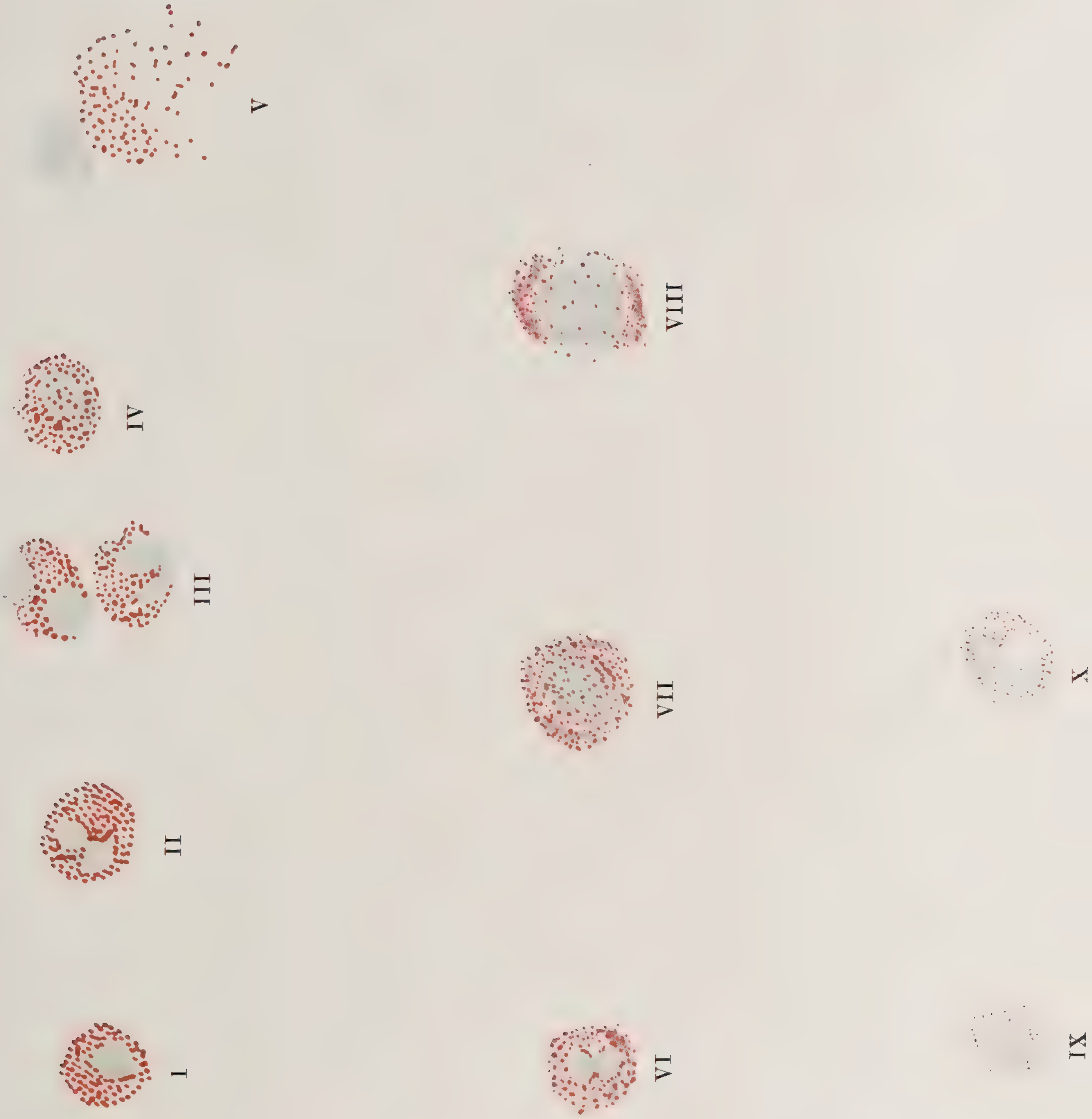
PLATE X.



Nos. I–V, eosinophiles from favorable cases of moderate scarlatina; Nos. I–IV, showing coarse granules crowding the cells; No. V, ruptured cell; Nos. VI–VIII, eosinophiles from malignant scarlatina, oxyphile granules having washed appearance; Nos. IX, X, polymorphonuclears with active oxyphile characters.

A HITHERTO UNDESCRIBED EPIZOÖTIC AMONG RABBITS AND RATS, CAUSED BY A FLAGELLATE MICROCOCCUS.[1]

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An epizoötic disease, causing many deaths among the experimental animals at McGill University, provided the material for this investigation.

Several forms of infection among rabbits are known to us, particularly the much studied hemorrhagic rabbit septicemia. This is plainly a disease affecting the respiratory tracts first, causing a hyperemia of the nasal mucous membrane with increased mucus secretion, which later becomes a purulent discharge. The lungs and pleuræ become involved and not infrequently there is a pericarditis and peritonitis. Death of the animals results in from four days to three weeks. Inoculated animals die in from twenty-four to seventy-two hours. The infective agent is a small bacillus with polar staining, which grows readily on ordinary media. This organism belongs to the fowl cholera group. The bacillus was first studied by Koch in 1878 who obtained it from infection produced by inoculating rabbits with putrefying meat. The biological characters are not given, but he described them as being oval cells with a long diameter of 0.8 to 1.0 μ. There is edema at the point of inoculation, the spleen becomes enlarged, and later the organisms are found in the capillaries of the various organs, especially the kidneys. Since then, the same organism has been isolated from other animals by different observers, hence the confusing nomenclature. It would seem to be identical with Pasteur's (1880) micrococcus of fowl cholera, and Flugge's Bac. cuniculicida. Subcutaneous injections of small quantities produce a septicemia in chickens, pigeons, geese, ducks, and also in rabbits and mice.

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[1] Read at the fourth annual meeting of the American Association of Pathologists and Bacteriologists, April 2, 1904, at New York.

At the point of inoculation there is edema and pus formation, and rabbits, which are very susceptible, die within a short time. Inoculations into the cornea of the eye cause a general infection and death of the animal.

In 1887 Theobald Smith described a microörganism as the cause of an epizoötic septicemia among his laboratory rabbits. Thoinot and Masselin described the same organism in 1888. In this disease the serous membranes are particularly attacked, along with congestion of the alimentary tract. The organism is very similar to that of fowl cholera and presents similar bi-polar staining. In the blood it appears in the form of diplococci, sometimes in short chains.

Eberth and Schimmelbusch found, associated with a disease among ferrets and wild rabbits, an organism similar to the one described by Smith. This organism, too, presents a certain similarity to that described by Koch. The organism is motile, shows bi-polar staining, and is negative to Gram. Chickens are not susceptible. The same organism was later observed by Eberth and Mandry in 1890, and also appears to be identical with the organism studied by Billings in America in 1888. Recently Catterina has described a motile micrococcus which he obtained from rabbits. The organism he found usually appeared singly and only seldom as a diplococcus. A darker portion was visible in the center, which at times was elongated, bacillus-like, and this he considered to be the nucleus. Catterina describes the organism as provided with two flagella. This organism caused no change in milk media, even after several days' incubation. Subcutaneous inoculation of his micrococcus in rabbits caused death within forty-eight hours. Examination post-mortem showed an enlarged spleen and brown appearance of the blood. The organism was also pathogenic for guinea-pigs and mice, causing death within sixty hours.

Epizoötic diseases among rats are much less known. Of late there has been much written of rats as carriers of the plague. It has been definitely shown that rats are subject to a disease similar to the pest, and caused by the bacillus of that disease.

In 1900 Danysz showed that the virulence of the Bac. typhi murium might be increased by passing the organism successively through a series of rats. His purpose was to obtain the organism of such virulence that it might be used as a means of destroying the house rat.

In 1903 Toyama described an organism pathogenic for rats and mice in Japan. It appears singly or in pairs, is larger than ordinary cocci, and negative to Gram. The chief lesions are in the spleen and the lymphatic glands, from which the organism is readily obtained.

The Montreal epizoötic. — The epizoötic which is the subject of this investigation began in the middle of October, 1903, when some fifteen rabbits died within two weeks. As the epidemic had gained quite a foothold, there was plenty of material to thoroughly investigate the cause of the outbreak.

The animal-house is a recently built brick structure of two stories, with a cement pavement on the ground floor. There are no separate rooms on either floor, but the animals are kept in rows in heavy wire cages with double bottoms. The building harbors some three hundred rabbits and seventy-five guinea-pigs, besides dogs, cats, rats, etc. As soon as any disease is noticed among any of the animals, they are removed from the animal-house to cages in the laboratory for observation and study.

Shortly after admitting the first animals to the new building, several white rats were sent in from the animal room in the medical building. The rats did not appear healthy, being thin and inactive. The hair on the body was uneven, and in places showed thinning out, even, in places, to complete baldness. These rats were placed in the common room on the first floor occupied by the rabbits and other animals. Such is thought to be the origin of the epizoötic.

Since then all the white rats have become similarly affected and died. The last two were sent to the laboratory, where they were kept under observation. The animals were particularly listless and showed little inclination to feed. They died after some weeks, at the end of which time the fur was very ragged. The skin of the bald areas was normal in appearance. The examination of the blood during life revealed nothing. Nothing satisfactory was obtained in the bacteriological examination, which in the one case gave sterile tubes from all the organs, while in the other animal a large abscess over the bladder gave ordinary pus organisms. As shown by our later experience, these negative results were in all probability due to the employment of inadequate methods of inoculating the media.

The first rabbits dead with the disease were sent to the laboratory during the second week of November; after this for a few weeks one or more animals were sent in each day.

The disease is essentially chronic in nature, extending over three or four weeks or more after the appearance of the first symptoms. All cases did not end fatally; several of the older rabbits recovered after six or eight weeks. The fatal cases occurred within four weeks after the onset. There were no complications during the course of the illness.

The first symptom to be noted was a peculiar clipped appearance of the fur, due to the loss of the coarser hair in patches. The clipped appearance was most marked along the back and on the flanks. In places not only was there a loss of the coarser hair, but also of the finer fur, leaving the animal quite bald. The head and legs were rarely affected.

The animals then became emaciated to such a degree that they were merely "skin and bone." In this condition the animals showed no inclination to feed and were inactive. Further than this nothing was to be seen externally. Most of the animals affected were under a year old, but in several cases a doe with her litter all contracted the disease. Two rabbits, four and five months old respectively, became paralyzed in the hind legs after three weeks' illness. The paralysis was complete, and the animals moved about with the hind legs dragging after them. The animals survived the onset of the paralysis for two days.

These nervous symptoms are comparable with those produced experimentally by Blachstein with inoculations of certain amounts of certain cultures of B. coli and by Charlton.

The latter inoculated rabbits with colon bacilli of low virulence, for an extended period of time, and found that after developing a severe anemia, the rabbits became paralyzed, first in the hind limbs and then progressively ascending until death. Charlton also noted in his cases of sub-infection by B. coli that in several cases the liver was smaller, with a distinct fibrosis. He was inclined to regard the fibrosis as consequent upon a coccidiosis present in the same.

In the epizoötic here described the death rate was higher among those under nine months old, and of all the rabbits affected sixty per cent died (not including animals used for experimental purposes).

Morbid anatomy. — Post-mortem examination was made on twenty-one animals. A summary of a single autopsy will be cited, of a well marked case, so that recapitulation of the condition found in the other animals will be unnecessary.

The animal was a young white doe eight months old. It had first shown signs of illness three weeks previously, when it was noticed that the hair was falling out. The fur appeared shaggy and looked as if it had been clipped with the scissors. This raggedness of the fur was particularly noticeable on the flanks and down the back. The skin everywhere appeared soft and healthy. There was no external lesion. During the last week the animal became emaciated and would sit quietly in one corner of the cage all day. There were no symptoms of digestive or respiratory trouble. *Autopsy.*— Externally there was nothing noted except the "clipped" condition of the fur and the emaciation. The coarse hair pulled out readily and was not broken. There were no external wounds.

On section there was little or no fat in any portion of the body; the muscles were normal in appearance.

In the abdomen the peritoneum was healthy and the cavity contained no fluid. No acute inflammatory condition existed either in abdomen or thorax. The blood in the vessels and heart was not coagulated.

The liver was slightly smaller than normal and was lighter in color. (The color of the liver varied in different animals according to the interval between death and the examination. In cases where the examination was delayed some hours, the liver was a dark red.) Small size of the liver was seen in several cases, and in one animal, an old doe, the liver was markedly fibrous and granular in appearance. The increase in the fibrous tissue was most decided in the capsule and about Glisson's capsule throughout the organ.

The spleen was normal in size and color, or, if anything, a little lighter in color. It was never increased in size, nor was it dark in color, except when, as above in the case of the liver, the post-mortem examination was delayed some time after death.

The kidneys were normal in appearance and size. There was very little perinephric fat.

The intestines showed nothing of note; the serosa was not injected; the contents were normal.

Pleural cavities, heart, and lungs showed nothing unusual, in all cases appearing healthy.

Bacteriological examination. — Cultures were made from the various organs and from the heart blood. The material was collected by means of sterilized pipettes from the interior of each organ. This was inoculated into fluid and solid media. Though positive results were obtained in some, the results were not found to be uniform, and were unsatisfactory. The method then adopted was to transfer, under strict aseptic conditions, a portion of each organ into flasks of nutrient broth. About fifty cubic centimeters of plain neutral broth were used for each organ. Great care was taken to sterilize all instruments used and to use fresh sets of instruments for each stage of the operation. Before dropping the organs into the flasks, the external surface was singed in the flame to ensure protection against external contamination. These flasks were then placed in the incubator at 37° C. and left for varying times of three days to two weeks.

In this way it was found that any living bacteria present in the organs selected had a better chance to develop than by using a small amount of the tissue juice.

Media were inoculated with heart blood obtained by extracting the blood from the right auricle with the pipette. Comparatively large quantities of liquid medium in flasks were employed.

Cultures were most frequently obtained from the spleen, next the liver, and rarely the heart blood. Cultures were never obtained from the kidney. Mixed cultures were most frequently obtained in growths from the liver. The mixed cultures contained, along with the micrococcus in question, B. subtilis and colon-like forms.

Cultures made from sixteen rabbits which presented the symptoms of the disease above described gave growths of a motile micrococcus in twelve instances.

Smears made directly from the blood showed the presence of the micrococcus in several cases. In not a few cases the same blood gave no cultures, a further evidence that the organism grows with difficulty when transplanted into laboratory media.

DESCRIPTION OF THE MICROCOCCUS.

The organism, a micrococcus larger than staphylococcus aureus, is seen most frequently in pairs, but also appears singly, rarely in short chains. It is larger than micrococcus agilis of Ali-Cohen. Sometimes it appears in regular clusters of four. Fresh specimens from the tissues show it in pairs, but not in chains. When grown on liquid media for several days an arrangement in fours is not infrequent. Within the various organs, by means of proper staining, the micrococci are shown to be in pairs.

They stain readily with the ordinary dyes, are not decolorized by Gram, and stain best with carbol-fuchsin and carbol-thionin. Staining for flagella by Van Ermengem's method, modified by Stephens,[1] demonstrates a single flagellum for each coccus, situated in the diplococcic forms at the pole opposite to the adjacent surfaces. At times two flagella arise from the same point on the one micrococcus. Each flagellum consists of a long, lash-like process, very slender, and in length many times the diameter of the organism. The flagella are stained with great difficulty and in only about one in six specimens were the flagella to be seen.

The flagella are so delicate that, even with the greatest care in preparing films, they were broken off and became entangled in the processes of other micrococci. The crossing of two or more of these flagella look not unlike branching of these processes.

No definite capsule could be demonstrated either about the diplococcus or in single cocci.

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[1] Thomson Yates and Johnston Laboratory Reports, Vol. 5, No. 1, 1903.

The coccus is motile, the movement being of a zigzag nature, in others it is a to-and-fro motion. But in either case the cocci do not cover much ground, and seldom do they shift the distance from the center to the periphery of the field under the microscope. Eighteen-hour cultures in a slightly alkaline blood serum broth were the most active and gave the best results for making films for flagella staining.

The methods used in determining the cultural characteristics are those recommended by the Committee of American Bacteriologists, except that the nutrient broth was prepared from beef extract, following Mallory's prescription (Mallory and Wright, Pathological Technique).

CULTURAL CHARACTERISTICS.

Nutrient broth. — Eighteen or twenty-four hour broth cultures show a diffuse cloudiness throughout. No pellicle is formed After forty-eight hours a grayish ropy sediment is produced which readily breaks up into fine flakes on shaking. *Blood serum broth.* — This medium was prepared by mixing equal parts of blood serum and glucose broth ; by sterilizing it in the Arnold steam sterilizer no precipitate is produced and the medium remains fluid. This was found to be the best fluid medium for growing the micrococcus, and in this it produced cloudiness in twelve hours. Motility in this medium was more marked than in plain broth.

Litmus milk is first turned red within eighteen hours, then decolorized and coagulated. The coagulum becomes firm within twenty-four hours or less.

Agar slant. — On a one and five-tenths per cent agar medium there is a luxuriant growth after fifteen hours. The colony spreads from the streak in a grape-leaf fashion ; the edges are smooth, wavy, and slightly raised ; the colony appears white and glossy and does not invade the medium from the surface.

Blood serum. — This is the optimum solid medium. The colony spreads from the streak as quite a thick growth. This is white, moist, and shiny, with smooth, even edges. Liquefaction of the blood serum takes place slowly — about the tenth day. Blood serum which has been slightly overheated, so that it has turned a grayish brown, is liquefied more readily — second or third day. Grown on a medium prepared by solidifying ascitic fluid, the colonies are small, round and isolated on the surface of the slant. Liquefaction takes place in three days. *Potato.* — Growth is slow on potato, being visible after forty-eight hours. After five days the growth is more luxuriant and appears as a yellowish, dry, spreading colony. The margins are irregular and "heaped up." *Gelatin.* — This medium consisted of a nutrient broth, slightly acid to phenolphthalein, with ten per cent gelatin added. Stab cultures made into the medium and grown at room temperature gave a granular growth along the line of inoculation. Towards the surface of the stab the growth is more vigorous with some spreading on the surface from the point of inoculation. Liquefaction takes place slowly and proceeds from above downwards in a funnel shape. On plates the colonies are small, grayish, with definite slightly raised margins. There is nothing characteristic in their appearance. At the end of a week the colonies would be seen sinking into small areas of liquefaction, though this latter process was slow. There is no odor from any of the tubes. In broth culture media containing the various sugars — glucose, lactose, and saccharose — there was no gas production. There was growth in the closed arm of the tube. *Temperature relationship.* — The organism grows best at 37° C., but also flourishes at room temperature, 20° C. Broth cultures resist a temperature of 58° C. for five minutes, but are destroyed when subjected to a heat of 60° C. for the same length of time. *Reaction of medium.* — The range of reaction of the medium varies from six per cent decinormal soda solution to four per cent decinormal hydrochloric acid solution. Beyond these limits the growth is very slight, even after several days. The best reaction for growth was found to be two per cent decinormal soda (to phenolphthalein). No growth was obtained in a medium with ten per cent decinormal soda or ten per cent decinormal hydrochloric acid.

Active cultures were still obtained from eight weeks old colonies which had dried, and by making daily transfers on to Lœffler's blood serum or in blood serum broth the activity was found increased. *Anaërobic cultures.* — The organism flourished when grown anaërobically in Buchner's jars, on Lœffler's blood serum and on agar slants.

Microscopical examination of the organs. — Portions of the various organs were preserved at necropsy, fixed in Müller's solution for an hour, and then passed through four grades of alcohol for hardening. The tissue was put up in paraffin blocks and cut. Sections were stained in hematoxylin and eosin, Van Gieson, and carmine. For demonstration of the bacteria within the tissue the sections were treated with carbol-thionin for three minutes and then decolorized to a faint blue with acetic acid solution 1–100. This latter method gave beautiful results, the organism appearing as diplococci within the tissue cells, while the granules of the cells had disappeared under the action of acetic acid. To verify the presence of the diplococci, the sections were also stained by Gram's method with a positive result.

The presence of living bacteria in the organs of healthy animals has been pointed out by Ford, but the bacterial flora is fairly limited to each species of animal. Thus in the rabbit Ford found that, beyond varieties of colon bacilli, occasional colonies of B. subtilis and B. mesentericus were encountered in cultures from the different organs.

Stained with carbol thionin, the liver showed the greatest number of organisms. These were present mostly as diplococci and were stained a deep blue. There was no special arrangement of the organisms within the liver cells — none were found intranuclear. Only rarely were any organisms found in the connective tissue or along the blood vessels. In some cases the liver cells were packed with these organisms, but all did not stain uniformly, a great many taking the stain only in part and appearing in a more or less granular condition. Others among these were distinctly dark blue, presenting similar staining qualities to the organism outside of the body.

Comparing the sections with those of a normal rabbit, I found that in the latter some of the liver cells did contain a small amount of granular material in their protoplasm and these granules resembled the poorly stained particles seen in the cells of the first liver. These granules had been studied by Adami in human cirrhosis of the liver, occurring also to a greater or less extent in the cells of most if not all the normal livers, and represent, in his view, the phagocytosis of these cells. In the normal rabbit's liver I could find no evidence of well stained diplococci which would bear any resemblance to micrococcus flagellatus. Stained by Gram's method, I was able to stain the micrococcus in the liver cells. On the other hand, the numerous granules which no doubt represent the broken down remains of these and other bacteria, similar to those described by Adami in Pictou cattle disease, were decolorized and could be recognized by the yellowish-brown color they assumed.

The spleen showed a fair number of these organisms, but in no wise in as large proportion as that in the liver. In the spleen the organisms bore no relationship to the parenchyma, they being scattered throughout the tissue outside the cells. In the spleen, differing from the liver, the organisms were not found in nearly the same abundance, nevertheless those which were present were well stained, nor did I observe any remains of bacteria as were found in the liver. Here, also, I was able to stain the organisms in the sections by Gram's method.

Although sections showed the micrococcus present in greatest numbers in the liver, yet it was difficult to obtain cultures from this organ. Cultures were more readily obtained from the spleen. Whether this is due to a stronger phagocytic action on the part of the liver cells is a point which I hope to determine in a subsequent study. By obtaining very thin sections and with proper staining methods the microörganisms or abundant diplococcoid remains were also demonstrated in the kidney. These lay, without exception, in the intermediate zone lying crowded in the cells of the tubules. All the tubules in the region did not show the organism, a great many of them being absolutely free. The cortex and the Malpighian corpuscles showed no bacteria nor were any seen in the tubules of the pyramids. Such bacteria as seen in the intermediate zone, when stained with Gram's, took on a brownish color and appeared as if they were the remains of dead organisms. They certainly did not take the stain like those in the spleen or the better stained organisms in the liver. This would agree with the bacteriological findings, no cultures of the organisms having been obtained from the kidney. In certain cells in the intermediate zone these remains were so crowded that, under the low power, the section took on a brownish tint.

Histologically no changes are seen in the spleen, kidney, heart, or lungs. In three instances the liver showed definite evidence of fibrosis following the portal system. The fibrosis was well marked in one instance in which the organ was distinctly smaller, with areas of fibrosis penetrating its substance. None of these livers showed coccidiosis.[1]

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[1] Since writing this paper another rabbit, a full grown adult, has come under observation, with the chronic progressive form of the disease. Its liver showed pronounced cirrhosis with, in addition, a very definite hemochromatosis — the first example, if I mistake not, of this condition in animals of the laboratory. Both liver and spleen gave cultures of our organism.

Sections of the skin from various portions of the body and the bald areas showed nothing of interest. No microörganism could be demonstrated in this tissue, although it is to be noted that the organism was isolated from cultures made from the hair on three occasions. Sections made from the spinal cord in the case of the rabbit which developed paraplegia showed an extensive hemorrhage in the substance of the cord at the region of the lumbar enlargement. The hemorrhage was not confined to the anterior horns alone, but was diffused irregularly on both sides. No organisms could be demonstrated in the hemorrhagic areas in the cord.

Inoculation of animals. — Four rabbits were inoculated intraperitoneally with two cubic centimeters of a twenty-four-hour broth culture of the micrococcus. The inoculations were repeated for three successive days. Of these animals, three completely recovered. The fourth died at the end of seven weeks, and showed slight symptoms of the disease above described. The same ragged condition of the fur was to be noted, but not at all marked. Sections made from the liver of this rabbit showed a condition similar to that seen in the diseased animals. There was an early stage of fibrosis, while the whole liver was slightly smaller and appeared granular. Proper staining also demonstrated the organism within the liver cells, both as well-stained bodies and in the granular, disintegrated condition.

Subcutaneous inoculations do not cause abscess formation. In another instance a rabbit was inoculated with five cubic centimeters of a twenty-four-hour broth culture intraperitoneally, and the rabbit killed on the following day. The peritoneal fluid of the animal was inoculated into a second rabbit and likewise, on the following day, the peritoneal fluid of the second animal was inoculated into a third. The third animal died at the end of five days and cultures were obtained from it similar to the organism inoculated.

Two guinea-pigs, each inoculated once with two cubic centimeters of twenty-four-hour broth culture intraperitoneally, died exactly fifty days after the day of inoculation. After death the microörganism was again isolated from the liver and spleen. When the organism was passed through several guinea-pigs, death was produced within thirty-two days.

Agglutination of the organism.— The blood was obtained from animals showing definite symptoms of the disease, and the serum was added to a twenty-four-hour broth culture of the organism. The highest dilution of the serum obtained from any animal ill with the disease was one to forty. The most frequent typical agglutination was obtained with a dilution of one to twenty. In animals inoculated with increasing doses of a broth culture a serum was obtained which gave an agglutination of one to eighty. Normal serum from rabbits had no effect on the organism in dilutions above one to eight.

SUMMARY.

We have here a disease, epizoötic among rabbits and rats, hitherto, to my knowledge, undescribed.

The disease is essentially a chronic one, ending fatally in over half of those affected. It affects chiefly young animals and is characterized by the falling out of the hair, anorexia and marked wasting. The duration of the attack is from four weeks to two months.

The specific organism is a motile micrococcus, to which the name of Micrococcus flagellatus is given. The micrococcus appears most frequently in pairs and a single flagellum is attached to the free pole of each coccus. Sometimes several flagella are attached to a coccus.

The organism can be readily isolated and cultivated from the spleen and also from the liver. Histologically it can be demonstrated in greatest abundance in the liver, where changes are produced not unlike a mild form of cirrhosis. The organisms found in the kidney are dead and represent only the remains of previous living bacteria. In the spleen the micrococci are most readily stained, showing that they are in a living condition, which fact is also borne out by greater ease in isolating them from the spleen than from the other organs.

In vitro the organism very quickly becomes attenuated, but may be increased in virulence by passage through a series of susceptible animals.

[This work was undertaken at the suggestion of Dr. J. G. Adami, to whom I am much indebted for valuable advice during its progress, for which I would here express my thanks.]

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ON A BACILLUS ISOLATED FROM WATER AND AGGLUTINATED BY HIGH DILUTIONS OF TYPHOID SERUM.[1]

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A review of the very numerous articles published upon typhoid agglutination shows that, very early in the study of this subject from a diagnostic standpoint, it was recognized that the blood of typhoid patients or of animals inoculated with B. typhosus would, if not adequately diluted, cause clumping of other organisms which were found to belong broadly to the colon group. But the standard of what constitutes adequate dilution has steadily advanced. The earlier observers held that a ten or fifteen fold dilution was adequate to separate off the typhoid bacillus from all other bacteria. As the hyper-immunization of animals against B. typhosus became more common, and blood serum was thus obtained capable of agglutinating typhoid bacilli when diluted one thousand fold or more, this limit was found inadequate. With increased specific agglutinative power on the part of the serum, other bacilli were found to become increasingly sensitive. But, apart from this, and dealing with human typhoid serum alone, the minimal dilution for differential purposes is now generally given as one of forty, some even (Stern) recommending one of fifty. Even this is not wholly satisfactory. Widal and Nobécourt in 1897 recorded a case in which, during typhoid convalescence, the serum of the patient agglutinated B. typhosus in a dilution of one in twenty, whereas a paracolon bacillus, isolated from the same patient from the pus of an abscess, was agglutinated by this same serum in a dilution of one in twelve thousand. In 1898 Stern called attention to the fact that the serum of typhoid patients frequently agglutinated forms of B. coli isolated from the stools of typhoid patients, sometimes in dilutions higher than those which caused clumping of the typhoid bacillus. He quotes other observers who had noted the same fact.

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[1] Read at the fourth annual meeting of the American Association of Pathologists and Bacteriologists, April 2, 1904, at New York.

The above cases, it will be observed, have this in common, that the forms agglutinated had been isolated from the patients affording the typhoid serum and the results might be explained purely as due to double infection.

Another series of cases are those in which the blood of hyper-immunized animals has been employed. Thus Beco in 1899, employing sera which agglutinated typhoid bacilli in a dilution of one in one hundred thousand, found that these would also agglutinate sundry strains of B. coli (some of them from typhoid stools, others out of normal stools) in a dilution of one in ten thousand. The same sera often caused agglutination of B. fluorescens liquefaciens and of the proteus obtained from typhoid and from normal stools respectively in the dilution of one in ten thousand and of one in one thousand. There was agglutination here, but it will be seen with much more concentrated sera.

The question of reciprocal agglutination has been attacked from a clinical standpoint by Durham. He found that, in an epidemic of gastro-enteritis associated with the presence of a variety of the B. enteritidis (Gärtner), the serum from eighteen out of the twenty-nine cases gave a positive reaction of 1 in 100 with the specific organism isolated from the liver of one of the cases. Five of these gave a minimal reaction at 1 in 100 to a typhoid culture, while, with a dilution of 1 in 10 or 1 in 20, five more gave a well-marked clumping. In another series of experiments he found that the B. typhosus had only a slight mutual serum reaction with B. enteritidis (Gärtner) and practically none with a Gärtner-like bacillus (bacillus "Os"). At the same time the Gärtner and "Os" organisms reacted mutually at 1 in 200. He nevertheless lays down that, below and including dilutions of sera of 1 in 100, it is immaterial whether true typhoid or Gärtner bacillus be used to obtain the typhoid reaction.

As Durham is dealing with a human infection in all his cases, it is difficult to draw any conclusions as to the multiplicity of the agglutinins present in the sera. It is not difficult to imagine that, having typhoid or typhoid-like lesions in the intestines, mixed infections may be liable to develop, each infection producing its own series of agglutinins.

Working along similar lines, Stern also employed human serum. He isolated four strains of the B coli, two of them from the diarrheic stools of typhoid patients, one from a case of enteritis in a suckling infant, while a fourth was from a case of catarrhal icterus. He found that the blood serum of twenty-five healthy men and patients suffering from diseases other than intestinal gave positive reactions with the organisms ranging in dilutions of 1 in 15 to 1 in 60. Many sera which do not give a trace of reaction with the typhoid bacillus at 1 in 10 are positive with B. coli at 1 in 40 or 1 in 50. The blood serum of eighteen typhoid patients agglutinated the coli strains above mentioned in the majority of cases in greater dilutions than was the case with the blood sera of non-typhoidal patients. The latter gave positive reactions with a maximal dilution of 1 in 60, while only three of the former were below this dilution. The rest were between 1 in 70 and 1 in 300.

There is thus, it would seem, a gradation in the reactive powers of various species of bacilli to typhoid serum. Some are wholly unaffected, others may be caused to clump when a very powerful serum is employed, others when a less powerful is used.

The observations of Rodet, judged by the observations of later observers, are somewhat difficult to classify. He states that, immunizing sheep against certain strains of B. coli and B. typhosus respectively, he obtained sera of such potency that the serum of an animal inoculated against B. coli agglutinated typhoid bacilli quite as powerfully as did the serum of another sheep which had been inoculated against typhoid, as again that the serum of a typhoid immune animal could agglutinate powerfully several varieties of B. coli. This is contrary to general experience. It may be that the author satisfied himself with a low grade of immunity and did not study the effect of high dilutions. It may also be that unwittingly Rodet encountered and employed, without studying the characteristic reaction in milk cultures, a form or forms belonging to the group about to be described. For there exists a group of organisms which is relatively highly susceptible to the action of typhoid serum. To my knowledge attention was first definitely directed to this group by Carl Sternberg in 1900. Sternberg's very full and careful paper has received relatively little attention.

In the course of an investigation upon an epidemic of typhoid fever in the suburbs of Montreal, a form very similar to those described by Sternberg was isolated by Drs. Adami and Chopin. It has seemed worth while to study it somewhat fully so as to call renewed attention to the existence of this type of organism.

The methods employed have already been described in a separate paper. Here it need only be said that, from two separate waters, pure cultures of this particular organism were isolated, both by the method of Cambier and that devised by Drs. Adami and Chopin. One of these was a tap water, the other, the supposedly polluted water of the St. Lawrence river, obtained at a point above the in-take, but below the drain, from which pollution is supposed to have taken place. This form was found to exhibit complete agglutination when diluted sixty times with three separate human typhoid sera, that is, the organism underwent complete clumping beyond the limit generally accepted as differentiating typhoid from other bacteria. The sera employed, it should be added, had been kept in the laboratory for several weeks, a little carbolic acid having been added. At first, whether because the organism was then more sensitive, or whether solely because the serum employed was not specially active, no distinction could be made between the clumping action of these sera upon this form and upon cultures of B. typhosus respectively.[1] Later, when the organism came into my hands for study and more powerful sera were employed, the difference in susceptibility could readily be distinguished. The organism was found to become completely agglutinated by the serum of a typhoid patient when this was diluted twelve hundred and forty times. The same serum, when diluted six thousand two hundred times agglutinated the typhoid bacillus. As will be pointed out, a similar susceptibility, and similar difference in degree of susceptibility, was made out when the blood serum of immunized rabbits was employed. Before, however, discussing in detail the agglutinative properties of this organism, it will be well to give a fairly full description of the same along the lines laid down by the Committee of American Bacteriologists.

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[1] Employing also the serum of a rabbit immunized against the B. typhosus, Dr. Adami and Dr. Chopin found no difference with dilutions of one in five hundred.

Source. — From tap water obtained in the township of St. Henri, as also from the region of supposed pollution of the river St. Lawrence at Verdun. *Morphology.* — From agar cultures, forty-eight hours' incubation: short bacilli, in pairs at times, also seen in long filaments or threads. Broth cultures twenty-four hours — short, stubby bacilli, rounded ends, uniform staining, no threads seen. *Size.* — 1.2 to 2.5 microns in length; breadth, 0.3 to 0.5 microns. *Stain* readily with ordinary aniline dyes. Are negative to Gram. Stain uniformly throughout. *Capsule.* — None observed. *Spores.* — None observed. *Vacuoles.* — None observed. *Motility.* — Is observed; sluggish, often rotatory, most pronounced in eighteen hours. Broth cultures much less active than B. typhosus. Cultures from one colony obtained from the Verdun water showed motility as active as that of B. typhosus. This colony was passed through the similar tests and showed no differences from the form here described as regards fermentation, acid production, etc. Flagella are abundant, but variable, so that we have found difficulty in making an exact estimate; forms (in a twenty-four-hour growth) having eight and ten flagella are very common, above ten are distinctly rare. *Growth.* — At 37° C. is most active, good growth also obtained at 20° C.

BIOLOGICAL CHARACTERISTICS.

Gelatine plate colonies. — Shape, round, with clear-cut margins; size, vary from two to five millimeters; margin, defined, smooth; texture, shiny, moist, fairly thick; color, yellowish by transmitted light, creamy by reflected light. *Agar plate colonies.* — Shape, round, flat, thin; size, two millimeters; margin, well-defined, smooth, even; color, transmitted light, bluish; reflected light, gray; texture, thin, glossy, moist. *Gelatine tube (puncture)*.— Granular growth down whole depth. No liquefaction at end of seven weeks. Colony spreads slightly on surface of stab. *Agar tube (slant cultures)*. — Growth along streak with a thin bluish colony spreading from it. Margins wavy. Transmitted light gives to colony a bluish appearance. Grayish color by reflected light. No change in medium.

Broth tube. — Tube clouded in eighteen hours at 37° C., no sediment forming until seventeen hours. On shaking, the sediment breaks up into stringy masses. No change in color of medium. No scum is formed. *Litmus milk.* — No visible (or at least very slight) change in twenty-four hours at 37° C. Acid is produced slowly, milk becoming a dark purple after four days. At the end of eleven days milk becomes slightly darker again. No coagulation of the milk in seven days. Variable coagulation in twenty days. There is never a firm clot, but milk becomes gelatinous at the bottom of the tube. Milk not decolorized. No odor. *Hiss cultures (plates).*—Twenty-four hours' incubation at 37° C., small white, fairly-defined colonies, the largest two millimeters in diameter, edges regular. With low power lens the colonies appeared with yellow centers and lighter margins. During the first week after isolation the colonies had quite a different appearance on this medium. They then were spreading and thinner, with a distinct tendency to thread formation, so that they could not at first be distinguished from control plates of the typhoid bacillus. With furthur growth these characters disappeared. *Elsner's medium.* — On this from the first the colonies in the main resembled those of B. coli rather than B. typhosus. It was noted, however, that when the medium was in a thinner layer and became somewhat dry, certain colonies had a dew-drop appearance characteristic of B. typhosus. Subcultures from these, however, did not maintain the same characters and were not distinguishable from subcultures from other more active growths. *Sugar broth.* — Glucose broth (one per cent). Abundant gas formed in twenty-four hours at 37° C. along with acidity. No pellicle formed. Tube clouded in closed portion. Lactose broth, ditto. Saccharose broth, ditto.

It is to be noted that, when first inoculated, it was only upon glucose broth that gas in small amounts was present at the end of twenty-four hours. Only after forty-eight hours did gas show itself in one per cent lactose and one per cent saccharose broth respectively. In subsequent cultures gas developed freely from all these sugars within twenty-four hours. Reference will be made later to temporary loss of gas production after pasaage through animals. *Potato.* — Growth occurs, shiny, creamy, yellowish. The film is thin and looks watery. *Blood serum.* — Growth similar to that on agar. Is not liquefied. *Indol.* — Not produced in first ten days. Formed after two weeks. *Pigment.* — Not produced. *Neutral red plates.* — Decolorized after forty-eight hours. *Neutral red tubes with glucose.* — Gas production with fluorescence and later (four days) decolorization to yellow color, only at bottom of tube. *Neutral red broth.* — Dextrose neutral red broth, fluorescence, yellowish to red.

PATHOGENESIS.

Animal.	Inoculation.	Result.
Guinea-pig I........	Inoculated with 0.2 ccm. of twenty-four-hour broth culture	Recovered.
Guinea-pig II.......	Inoculated with 0.7 ccm. of twenty-four-hour broth culture	Recovered.
Guinea-pig III.	Inoculated with 1.0 ccm. of week old culture.....................	Died in forty-eight hours.
Guinea-pig IV.......	Inoculated with 0.75 ccm. of broth culture gained from peritoneum of rabbit......................	Died in twenty hours.
3 Rabbits	Inoculated each with 1.0 ccm. of forty-eight-hour culture.........	Died in eighteen hours.

At the autopsies on the animals that died the peritoneal cavity was moister than usual and the intestines showed injection and somewhat watery contents. No other definite changes were noted.

From the above description it will be seen that the bacillus resembles B. typhosus in size, morphology, staining reaction, growth on gelatine, agar, and blood serum, non-liquefaction of gelatine, absence of scum in broth cultures; feeble production of acid in litmus milk, and lack of coagulation.

It differs from that organism in motility, growth on potato and on neutral red media, on Elsner's medium and, in its later growths, upon Hiss plates, as also in the eventual production of abundant gas on glucose, lactose, and saccharose. It is distinguished from B. coli (B. communior, Ford) more particularly by its growth in litmus milk, lessened acid production, and absence of coagulation and again by its agglutinative properties.

A puzzling feature was the relative slowness with which certain of these features were developed. Unfortunately we did not follow the recommendation made by Fuller and Johnson and neglected to make preliminary growths for a week upon gelatine, agar, and broth before beginning to study the characters of the organism. Possibly this would have eliminated some of the variable features. But it is worthy of note that the indol reaction only showed itself at the end of twenty days' growth; that the colonies on the Hiss plates were at first distinctly of the typhoid type, though later they came to resemble those of the Coli group, and that at first only glucose was fermented and that slowly. In all these points the bacillus, when first studied, showed a closer approximation to the typhoid bacillus. Regarding this last feature, namely, the fermentation of different sugars, it is noteworthy that, after passage through the rabbit, cultures showed a similar temporary loss of fermentative powers, recovering these only after several days.[1] These variations in properties are at least suggestive of the existence of a form or forms of somewhat unstable characters intermediate between the colon and typhoid bacilli. This impression of instability is not lessened by studying Sternberg's description of his organisms.

COMPARISON BETWEEN THIS ORGANISM AND THOSE ISOLATED BY CARL STERNBERG.

Sternberg describes a series of five organisms, three of which were isolated from waters in the neighborhood of Vienna, the other two being laboratory stock cultures previously isolated from water. All of these showed themselves peculiarly sensitive to typhoid serum. A typhoid serum which agglutiuated a stock typhoid bacillus completely in dilutions of 1 in 1,000, and partially when diluted to 1 in 1,400, agglutinated his five strains as follows:

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[1] A lethal dose of the bacillus was inoculated into the peritoneal cavity of the rabbit and immediately after death cultures were obtained. The appearance of the organism and the cultural characteristics were now those of the bacilli inoculated, except that, in the fermentation tubes, there was a very slight development of gas in glucose broth, none in lactose or saccharose. Transfers were made from these tubes into the respective sugar broths, that is, the glucose colony was transferred to glucose broth, the lactose colony into lactose broth, etc. After twenty-four hours' incubation, there was an increased amount of fermentation in the glucose tube, but still none in the lactose nor in the saccharose. A second transfer was made similar to the above and now, at the end of another twenty-four hours, glucose and saccharose were both fermented, no gas appeared in the lactose. In the glucose and saccharose broths there was acid production. In the lactose broth no change was evident, though there was growth in the closed arm of the tube. Four days' incubation of the organism in lactose broth gave a smaller amount of gas formation and transfers from this again into lactose broth led to its fermentation in twenty-four hours. The stock culture, as control, produced gas from all these sugars in twenty-four hours.

XB.; Complete agglutination with dilutions of 1 in 1,400.
WL.; “ “ “ “ “ 1 in 1,000.
Kas.; “ “ “ “ “ 1 in 1,000.
C 12; “ “ “ “ “ 1 in 600, partial agglutination 1 in 1000.
C 14; Complete agglutination with dilutions of 1 in 600, partial agglutination 1 in 1,000.
B. typhosus; Complete agglutination 1 in 1,000, partial 1 in 1,400.
B. coli; No agglutination with dilutions of 1 in 600.

From this it will be seen that three of these organisms were at least as sensitive to typhoid serum as was B. typhosus, and one of them, KB., was even more sensitive.

Four of these strains (KB., WL., C 12, and C 14) were culturally very closely allied, differing but in minor details.

WL. alone was found pathogenic for guinea-pigs and that but feebly. The form Kas. differed from all the rest in that it gave invisible growths on potato, while further the growths on broth were clear and not cloudy, and gave a slight sediment at the end of twenty-four hours.

As is so frequently the case with allied forms of bacilli of the colon-typhosus group, a study of Sternberg's detailed tables of the characters of the various forms, and comparison with the tables here given, brings up the problem of what are and what are not to be regarded as specific differences. So far as his description permits us to arrive at a conclusion, — and it is, on the whole, full and clear, — his forms, with the exception already noted (Kas.), differ from what are here under discussion and differ among themselves only in the following points:

(1) *Size and shape.* — All of Sternberg's bacilli were short rods, C 14 so short as to be coccus-like; our organism in certain cultures was quite as long as B. typhosus. But it is to be noted that KB. and WL., like our form, were apt to form filaments.

(2) *Motility.* — KB. and C 12 are described as motile. WL. and C 14 as sluggish; our form as moderately motile.

(3) *Polar staining.* — WL. showed this on agar (?) and, together with KB., showed it on potato. Our organism never exhibited it.

(4) *Growth in broth.* — C 14 formed a scum after six days. All the others were negative in this respect.

(5) *Growth on potato.* — This in the case of KB. and WL. was very dense.

(6) *Coagulation of milk.* — None of these forms coagulated milk, but C 12 and our organism caused some thickening (at times) of the milk.

(7) *Acid production.* — This was slight in all cases save in that of C 12.

(8) *Pigment production.* — WL. developed a brownish-yellow color in gelatine colonies. All the others apparently only took on a faint yellow tinge.

(9) *Indol production.* — This was negative in all Sternberg's cultures; with our organism showed itself at the end of two weeks. (It is possible that Sternberg did not continue his observations over this length of time.)

(10) *Odor.* — The cultures of C 12 alone had a foul odor.

(11) *Pathogenicity.* — All of Sternberg's forms save WL. were negative in this respect and WL. gave positive results only in one out of three guinea-pigs. Our form was definiteiy pathogenic both for rabbits and guinea-pigs.

I have here, I believe, indicated all the characteristics in which these forms showed any marked divergence. And it will be seen that each of these differences belongs to the debatable territory. Size and shape vary largely, according to previous environment. Of acid production and pathogenicity the same may be said. Any one who has worked with the Coli group knows the variability in the formation of a scum and the development of odor, as again in polar staining. And in this group pigment and indol production are notoriously variable. While studying this one organism, we have noticed variations in motility which were found hard to explain.

Recognizing the need of giving to a microbe having definite characters a definite name, — a name better than KB. or C 12,— I have had a strong inclination to name that here described the Bacillus Sternbergii, in recognition of Sternberg's pioneer work in the study of forms which agglutinate with high dilutions of typhoid serum. But it may be that further workers will determine that one or more of the points of difference here indicated are specific, that our form may come to be regarded, not merely as a variant, but as a species distinct from any of those described by that worker and so not as one of Sternberg's bacilli. Were I to name it B. Sternbergii that might only cause confusion, hence, as a name ought, I think, to be given, I denominate it *Bacillus perturbans*.

ON THE AGGLUTINATIVE REACTIONS OF B. PERTURBANS.

The agglutinative tests were all made macroscopically. The method used was similar to that described by Drs. Adami and Chopin. But, instead of using the massive cultures employed by them, half liter flasks were employed containing four hundred and fifty cubic centimeters of water and fifty cubic centimeters of plain nutrient broth which, after inoculation, were incubated at 37° C. A series of the long tubes described by them were then filled with ten cubic centimeters of the culture and to each was added a definite amount of typhoid serum. The method was found very convenient and the agglutination could be watched in a whole series of tubes at the same time, which cannot be done when the hanging drop is employed. The method is also much faster than that by the aid of the microscope and does not entail the tedious labor of the latter method.

In the first series of observations a relatively active serum was employed, taken from a case of typhoid fever in the third week. The following results were obtained:

TABLE I.

Agglutinative action of human typhoid serum on B. typhosus and B. perturbans respectively.

Tube.	Extent of Dilution.	Agglutination Noticeable in Fifteen Minutes.		Agglutination Complete in Three Hours.	
		B. typhi.	B. perturb.	B. typhi.	B. perturb.
1	Control	0	0	0	0
2	1–310	+	+	+	+
3	1–620	+	+	+	+
4	1–775	+	+	+	+
5	1–1,030	+	+	+	+
6	1–1,240	+	+	+	+
7	1–1,550	+	Trace	—	+
8	1–3,100	+	0	+	?
9	1–6,200	+	0	+	0
10	1–8,260	0	0	0	0

The striking feature of this experiment is the height of the dilution at which the agglutinative action was obtained for our bacillus. It should be added that the typhoid culture was an old laboratory strain presenting all the typical features of B. typhosus and reacting to this and other typhoid sera in high dilutions. B. perturbans agglutinated with this serum in dilutions of one in fifteen hundred and fifty.

It might be argued that the case of typhoid fever from which this serum was obtained was possibly one of mixed infection, so that the serum of the patient was not purely and simply a specific typhoid serum. To test this argument, two healthy rabbits were immunized against B. typhosus by repeated inoculations. The blood of neither animal, before inoculation, caused any sign of agglutination with either the typhoid or our bacillus in dilutions of one to sixty-two.

TABLE II.

Agglutinative action of typhoid immune rabbit serum on B. typhosus and B. perturbans respectively.

Tube.	Extent of Dilution.	Agglutination Noticeable in Fifteen Minutes.		Agglutination Complete in Three Hours.	
		B. typhosus.	B. perturbans.	B. typhosus.	B. perturbans.
Rabbit I.					
1	Control	0	0	0	0
2	1–62	+	+	+	+
3	1–124	+	+	+	+
4	1–155	+	+	+	+
5	1–310	+	+	+	+
6	1–620	+	0	+	+
7	1–930	0	0	Partial	+
8	1–1,240	0	0	+	0
9	1–2,480	0	0	Partial	0
Rabbit II.					
1	Control	0	0	0	0
2	1–124	+	+	+	+
3	1–310	+	+	+	+
4	1–620	+	+	+	+
5	1–930	+	0	+	+
6	1–1,240	+	0	+	+
7	1–2,480	0	0	+	+
8	1–4,960	0	0	+	0

The result of these three experiments indicates that a relationship does exist, so far as regards agglutination, between the typhoid bacillus and our organism when treated with immune sera. That is, both organs have affinities for a common immune serum, but these affinities exist in different proportions in each organism, thus giving us agglutination with different degrees of dilution. A difference was also noted in the time and amount of agglutination which was produced by the two organisms. In the stronger dilution, B. typhosus reacted almost immediately, giving a loose, flocculent agglutination which settled like flakes of snow. Even after twenty-four hours standing there was no change in appearance in these loose flocculi. The agglutination of B. perturbans, on the other hand, was in much more compact masses which, when they settled at the bottom of the tube, formed a dense granular sediment. The impression given was that the typhoid serum acting upon the typhoid bacillus led to rapid loose clumping with the formation of large masses, whereas the same serum, acting upon B. perturbans, caused smaller clumps which did not tend to fuse together as they fell or slowly sank through the medium.

The process was now reversed and rabbits were inoculated with increasing doses of our bacillus until a fairly potent serum was obtained. The potency of the serum was roughly tested twice a week by obtaining a few drops of blood from the marginal vein of the ear. When the serum became so powerful that it would agglutinate the homologous organism in a dilution of over one in fifteen hundred, the animal was bled and the serum collected.

TABLE IV.

Agglutinative action of perturbans serum on B. typhosus and B. perturbans respectively.

Tube.	Extent of Dilution.	Agglutination Noticeable in Fifteen Minutes.		Agglutination Complete in Three Hours.	
		B. typhi.	B. perturbans.	B. typhi.	B. perturbans.
1	Control	0	0	0	0
2	1–62	+	+	+	+
3	1–124	+	+	+	+
4	1–310	+	+	+	+
5	1–620	+	+	+	+
6	1–930	0	+	+	+
7	1–1,240	0	0	+	+
8	1–2,480	0	0	+	+

It is evident from these results that there is an interaction of the sera of the two organisms. The perturbans serum, though of comparatively the same potency towards its homologous organism, had a relatively much stronger agglutinative action upon B. typhosus than typhoid immune serum had upon B. perturbans. The amount of agglutination to be noted in the respective tubes of the typhoid bacillus and B. perturbans when treated with perturbans serum, was fairly equal even in the tubes with the maximum dilution. But, as seen in the previous experiment, the action of the special serum on a specific organism produced the reaction more quickly, and the flocculi were rather looser and not so granular as those of the other organism.

Here I would note that, in the course of a rapid and incomplete immunization of rabbits with B. perturbans extending over only ten days, Dr. Chopin did not obtain nearly so powerful results. The serum, while reacting with both organisms, was very definitely more active with B. perturbans, bringing it down almost immediately, whereas a precipitate was obtained with the typhoid bacillus only after three or four hours. The dilutions employed were one in twenty. These observations would seem to indicate that the homologous develops, as might be expected, more rapidly than the heterologous reaction.

Sternberg describes a somewhat similar series of studies in regard to his several microörganisms. As already stated, he found that there were differences in the clumping of the various forms with human typhoid serum. Inoculating a series of rabbits with pure cultures of the different strains, he found that their sera reacted variously towards cultures of the different strains, as also towards the typhoid bacillus. Thus the WL. rabbit serum gave a positive reaction with 1 in 100 to WL.; a reaction with 1 in 60 with Kas.; a reaction of 1 in 200 to B. typhosus. Kas. rabbit serum gave, in a like period, a positive reaction with 1 in 100 to Kas.; ditto of 1 in 200 with the typhoid bacillus; a negative reaction throughout to KB. KB. rabbit serum gave a positive reaction of 1 in 200 to KB.; positive with only 1 in 40 to Kas.; positive to B. typhosus with 1 in 100. The grade of susceptibility is not very high in these cases, but it will be seen that he obtained, not merely the same degree of reaction, but in two cases an even stronger reaction with the typhoid bacillus than with the homologous organism.

It might be expected that the maximal degrees of dilution of the immune sera producing agglutination of the two organisms should bear some relationship to one another. This, however, is not wholly borne out by the results obtained with our sera, nor in C. Sternberg's results just given. And again, when attempting to repeat this experiment with another strain of B. typhosus, I found that the same perturbans serum only agglutinated this after a maximum dilution of 1 in 930 (in place of 1 in 2,480).

This apparent discrepancy it is possible to explain along the lines of Durham's theory. Agglutinins are not specific nor individual entities, but, on the contrary, must be looked upon either as complex bodies or as a combination of several bodies, all of which may be satisfied by affinities of the homologous organism. Other organisms closely allied may possess one or more affinities for a like number of the agglutinative bodies present in that serum, hence we obtain agglutination to a certain degree of one organism by the agglutinating sera due to the employment of another. But it does not necessarily follow that the process can be reversed, for the affinities of the first organism may not be in a proper proportion to satisfy the bodies in the agglutinative serum of the second.

Durham has pointed out that similarity of cultural characteristics is no proof that there should be any relationship in the agglutinative qualities of closely allied organisms. Studying, for example, the enteritidis group, he was unable to obtain any clumping of B. "Gwyn" or Cushing's "bacillus O" by B. enteritidis serum; while, further, he found that the Gwyn serum acting on B. Gwyn up to one in 20,000 had not the slightest effect on Cushing's "bacillus O," although culturally the two organisms are scarcely distinguishable. He notes further that undistinguishable strains or races of B. coli communis do not react equally to colon sera of high potency.

I cannot pretend to have exhausted this subject of reciprocal agglutination; on the contrary, these represent but initial studies.

These observations so far serve to confirm the conclusions already recorded by more than one worker, namely, that even where a positive result is obtained with relatively high dilutions of typhoid serum, it is unwise, not to say mistaken, to regard the reaction as absolutely specific. Speaking practically, it is the presence of the reaction plus the clinical symptoms of the case that must be taken into consideration before a diagnosis of typhoid fever is regarded as reasonably established. And now that in so many quarters it is being shown that, with reasonable care, cultures of the typhoid bacillus are obtainable from the blood, we would suggest that such cultures afford the surest diagnosis. So also the conclusion is obvious that, in the examination of suspected waters, while methods based upon the phenomena of agglutination are most valuable in aiding rapid diagnosis of the existence of typhoidal contamination, the mere appearance of an organism which clumps with typhoid serum is inadequate as a test. No positive result can be gained until the bacillus in question is studied upon differential media.

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CARCINOMA OF THE BILE PAPILLA.

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Although cases of cancer of the bile papilla have been accumulating fairly rapidly in literature, the affection must still be considered of somewhat unusual occurrence. Moreover as each case presents certain features of its own, especially regarding its epithelial origin, I considered it a sufficiently interesting and profitable study to record the following two cases.

Before passing on, I wish to express my indebtedness to Dr. Martin and Dr. Bell, under whose service the patients were admitted to the Royal Victoria Hospital, for their permission and use of the clinical notes, and to Dr. Adami for the protocols of the post-mortem examinations.

CASE I.—V. R., male, æt 62, was admitted to the Royal Victoria Hospital under Dr. Martin, January 3rd, 1903. Patient was born in England and came to Canada at the age of 48. He worked as a mason until six years ago; since then he has been a gardener. There was a history of specific disease at the age of 18, and also a history of alcoholism. Family history was negative.

Present Illness.—On July 1st, 1902, patient noticed some enlargement of the abdomen, which went on until December, when he had some pain over the liver, accompanied by constipation. By January he was somewhat jaundiced. He had not worked for three weeks previous to admission to hospital, and during this time food caused some nausea and vomiting. Patient noticed that the stools were foul and "like putty." For the last two months the urine has been dark coloured and in this time he estimated he had lost twenty-five pounds.

Condition on admission.—The patient was somewhat emaciated, the muscles were small, the skin markedly jaundiced and dry. His intel-

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ligence was fair though at times dull and listless and occasionally irrational. There was dull pain all over the region of the liver, which was enlarged, its lower edge reaching as far as the navel. There was a condition of general arterial sclerosis, and a systolic murmur was heard at the apex with an increased aortic second sound. The abdomen was full, with some tenderness in the epigastrium. The urine was a dark amber colour, alkaline in reaction and showed the presence of bile. His blood count showed 3,470,000 red cells and 20,800 white cells with 50 per cent. hæmoglobin present. While he was in the hospital the jaundice steadily increased, and with it the patient became more stuporous. He was steadily on the downhill and died three weeks after admission.

Autopsy.—Body was that of an elderly, emaciated man, with skin of an intense yellow colour. There were slight external piles. The lungs showed a hypostatic congestion and on section were of a greenish yellow tinge due to bile staining. There was no sign of tuberculosis.

The pericardial cavity contained bile stained fluid. The heart was small, and its coronary arteries showed evidence of arteriosclerosis. The aorta presented some small patches of fatty degeneration. The viscera of the abdomen had a distinct yellow colour, while the cavity contained about 10 c.c. of yellow fluid with abundant lymph flakes. Some loose plastic adhesions were found between the coils of small intestine. The edge of the liver extended some 6 c.m. below the costal margin. The stomach walls were thin, of a slatey grey colour, but otherwise normal. The duodenum was fairly capacious and from without nothing abnormal could be detected. On opening it, the bile papilla was seen projecting as a firm even mass into the lumen of the bowel. A probe passed into the papilla was arrested, 1 c.m. from the orifice, and brought away with it some puriform material, which under the microscope consisted of columnar cells, and here and there collections of polymorphous cells of large size, closely resembling cancer cells. The remaining portion of the intestinal tract was normal. The liver was large, its surface smooth, except for a few sessile nodular projections over the upper anterior part of the left lobe, resembling nodular hypertrophy. The organ was distinctly soft and on section bile stained. Dilatation of the bile ducts was found everywhere and localized areas of necrosis could be seen along these. The larger bile ducts in the liver were the size of a lead pencil and contained fluid bile, while the common bile duct admitted the thumb. This distention of the duct was followed by a tight constriction immediately as this passed into the head of the pancreas. At autopsy it was impossible to pass a probe through the duct to the orifice on the papilla, but on dissecting the specimen it was found that the obstruction was due to pressure and kinking of the duct, rather than the invasion of the growth into the lumen. The pancreas showed marked atrophy and general dilatation of the main duct to the diameter of a lead pencil. A probe passed from the pancreatic duct was similarly arrested in the ampulla. The pancreatic lobules were surrounded by strands of firm connective tissue, and about the head there was some fat necrosis. No secondary growths were found and the remaining organs were normal.

Anatomical Diagnosis.—Columnar celled adeno-carcinoma of the Ampulla of Vater; obstruction with dilatation of the biliary and pancreatic ducts, icterus, acute plastic peritonitis, right hypostatic pneumonia, right old pleural adhesions.

Case II.—J. M., æt 40. Admitted to the Royal Victoria Hospital under Dr. Bell on September 24th, 1903. There was a history of typhoid fever eight years ago, and also of the use of alcohol to a moderate extent. Patient worked in the lumber camps during the winter, and was in perfect health until he returned in March, when he complained of a dull pain in the back. He was admitted to the hospital in June but remained only 18 days, with little improvement. The pain continued and made him feel weak. His appetite began to fail and he lost some thirty pounds between March and September. A week before admission to the hospital he vomited four or five times.

On admission to hospital, patient was poorly nourished with well developed but flabby muscles. Skin was dry and harsh with a yellow tinge. The conjunctivæ were distinctly yellow. A dull aching pain in the lumbar region, chiefly on the left side, radiated to the front of the abdomen and chest. As there was some tenderness over the umbilical region a thorough examination could not be carried out, but no mass could ever be felt. His appetite was poor, while he suffered from nausea with the sensation of weight in the stomach. He was constipated and the fæces were pale. The urine contained bile, but no albumen. The red blood cells numbered 3,910,000. While in the hospital the jaundice kept progressively increasing. He complained constantly of pain in the abdomen but could give no definite location of greatest intensity. An exploratory laparotomy was undertaken in October with the resulting diagnosis of malignancy situated about the head of the pancreas. Nine days after this, during a fit of coughing, the wound broke open, and another incision was made and the gall bladder was stitched to the edge of the second wound and drained. The patient continued getting weaker and died on November 12th, 1903.

Autopsy.—Body that of a tall, emaciated man, with a yellow colour to the skin. Two wounds of recent operation were situated over the site of the gall bladder and in the median line below the umbilicus respectively.

The lungs were healthy looking, except the right lower lobe, which showed evidence of terminal pneumonia and also a cluster of small abscesses along the anterior margin of this lung containing streptococci. The heart was small but showed nothing special. In the abdomen the small intestine was adherent to the recent median wound, and was covered with flakes of slimy lymph which yielded streptococci. There were about 350 c.c. of bile stained fluid in the abdominal cavity. In the duodenum, the site of the bile papilla was the seat of a shaggy necrotic ulcer measuring 3 x 2 c.m., into which the bile duct opened apparently without difficulty. The pancreatic duct opened 4 c.m. below the ulcer and admitted a probe readily. The common bile duct was tortuous, but a probe could be forced through it from above. The edges of the ulcer were somewhat undermined, leaving a continuous tag of mucosa overlapping it. The mucosa seemed uninfiltrated by any growth and was soft right to the edge of the ulcer. The liver was rather small, of a dark green colour with a deeper pigmentation in the centre of the lobules, giving the organ a dark green nutmeg appearance. The organ was firm with a smooth surface. Edges of the right lobe were sharp showing evidence of atrophy. The gall bladder was partially distended with dark fluid bile while the common bile duct was dilated and tortuous, and was constricted by several infiltrated glands along its course. The duct was patent as it passed through the duodenal ulcer, which appeared to be of the nature of a broken down primary cancer of the papilla. The common bile duct showed no involvement in the new growth, except at the lower end. The pancreas was relatively larger than normal, firm and fibroid, but at no point did it show the presence of secondary growth. The mesenteric glands in this neighbourhood and at the root of the mesentery were œdematous, but only a few along the bile duct had the appearance of being involved in secondaries. With the exception of having canalized thrombi in the iliac veins, and a left-sided inferior vena cava to the height of the left supra renal vein, the other organs were normal.

Anatomical diagnosis.—Cancer of the bile papilla with ulceration; secondary growths in the periportal lymph glands; obstruction and dilatation of common bile duct; icterus; aberrant orifice of main pancreatic duct; subacute purulent peritonitis; abscesses of lower lobe of right lung; aberrant situation of inferior vena cava; old canalized thrombosis of the iliac and pelvic veins; chronic interstitial nephritis with recent parenchymatous nephritis.

Before going on to the discussion of the pathological findings along with the microscopical examination of these cases it might be well to briefly run over the histology of the bile papilla as we normally find it.

Normal Histology.—The bile papilla represents the puckered up mucous membrane of the duodenum, where the bile duct opens in common with the pancreatic duct into the lumen of the intestine. It is situated on the left side, about the middle of the second portion of the duodenum, and projects slightly above the surface of the mucosa. Frequently on opening the duodenum, it escapes detection and is found only by passing a probe through it from the common bile duct. Normally the papilla is soft and is not unlike one of the valvulæ conniventes from which it cannot be distinguished by the feel of the finger. However, when it becomes involved in a new growth, or its lumen is occluded by a gall stone, it stands out as a hard mass into the lumen of the bowel.

The structure of the papilla is essentially that of the duodenum with the ampulla of Vater passing through its centre. The duodenal surface is covered by an epithelial layer continuous with that of the intestine. Villi are present, as are also the crypts of Lieberkühn, both of which are lined with columnar cells. The columnar epithelium on the villi does not differ from that found in the crypts, the object of both being to increase the surface area of the intestine. The muscularis mucosæ surrounds the papilla like an orbicularis muscle, and in a similar manner also surrounds the ducts of Brunner's glands, controlling their orifices with a valve-like action. The submucosa consists of firm connective tissue with glands which are found only in the duodenum. These duodenal, or Brunner's glands as they are called, are tubular racemose glands, whose branchings are coiled in the uppermost layer of the submucosa, while their ducts pass through the muscularis mucosæ to reach the intestinal surface between the villi. The ducts may or may not open into Lieberkühn's crypts. The lining cells of these glands are cylindrical, merging into a more cubical character as the duct passes through the muscularis mucosæ.

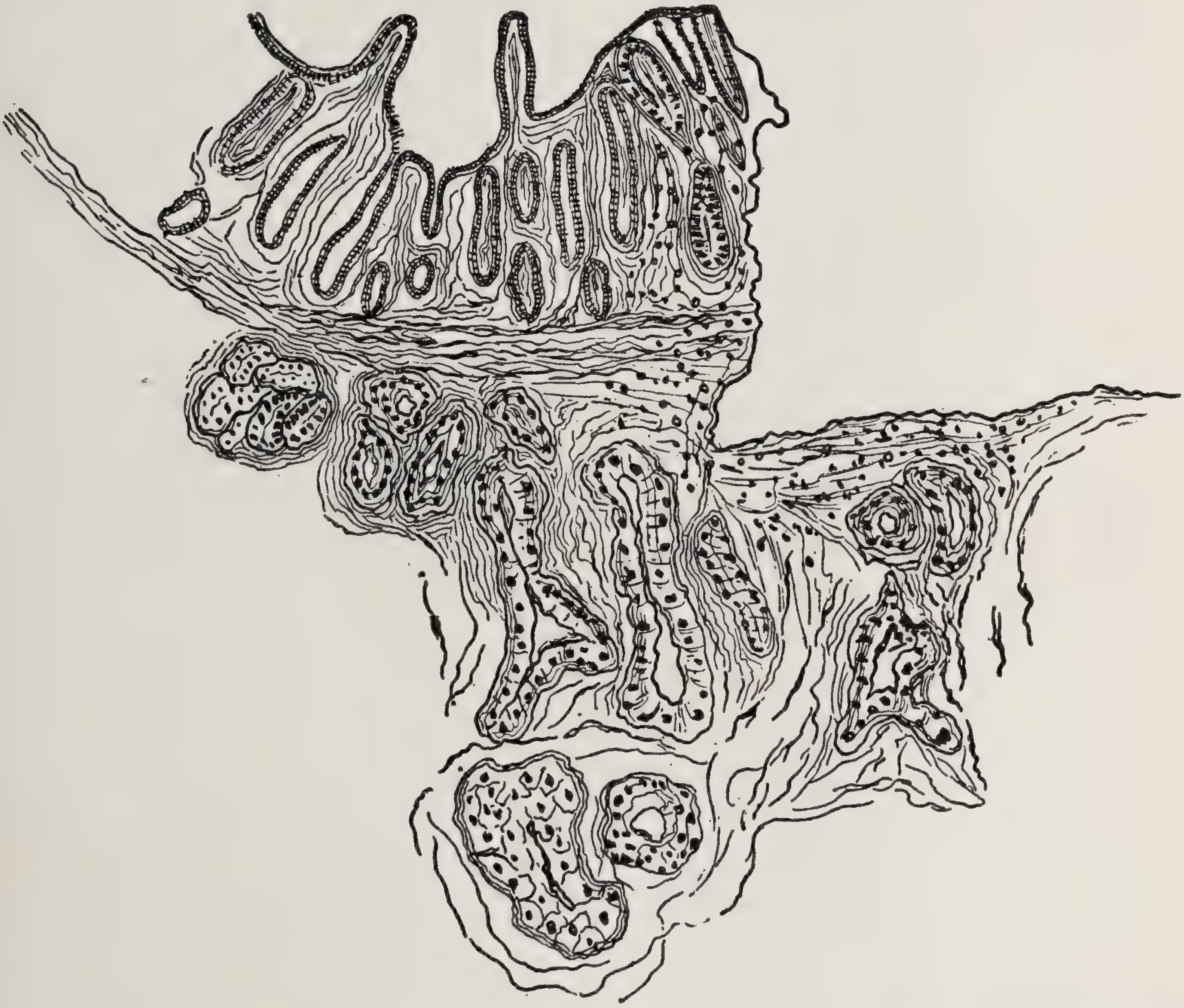
Brunner's glands are described as being present only in the upper part of the duodenum. I have found them present in and about the bile papilla in all of four cases I have examined. The muscularis and serosa of the duodenum do not differ from those found in the remaining part of the small intestine.

The ampulla of Vater, the small recess in the papilla, into which open the common bile duct and the pancreatic duct, measures 0.5 c.m. in length. The lining of the ampulla is continuous with, and does not differ from that of the bile duct, both being columnar celled.

Anatomical variations of the ampulla are not uncommon, and malignant growths complicating the anomalies set up a different train of symptoms for each. Thus at times we find the bile duct and Wirsung's duct unite at some distance from the papilla, converting the ampulla into a long tube; or else a septum exists between the two ducts throughout their course through the ampulla, when each opens by a separate orifice on the top of the papilla. Occasionally too, the ducts open into the duodenum at some distance from each other. In this case the bile duct opens at the normally situated papilla.

Microscopical Examination of the reported cases.—In the first case cited the microscopical examination showed the tumour to extend laterally from the epithelium lining the ampulla, for a distance of not more than one centimetre, and to infiltrate the muscularis of the bowel by solid columns of cells. The free surface of the tumour within the ampulla showed a partial degeneration and softening which accounts for the epithelial debris removed with the probe during the *post mortem* examination. There was very little change, if any, noticed on the duodenal surface of the papilla, other than a slight inflammatory condition. Brunner's glands could not be found involved—in fact, the growth did not extend as close to the surface as these glands, but was lying rather closer to the head of the pancreas where the cells became more spheroidal. No part of the pancreas was found to show any infiltration of the aberrant tissue, while the pancreatic duct was likewise free. The neighbouring lymph glands showed no secondaries, nor were any found in the liver, though in the latter organ there was an overgrowth of the fibrous tissue along the portal systems and an atrophied condition of the liver cells. The tumour is a primary cancer of the ampulla.

In the second case, sections taken from the region of the papilla, showed the structure of an adenocarcinoma. Near the surface it was more of an adenomatous character than is found in the deeper parts where the alveoli contain large multinuclear cells. In the sections the growth can be traced as extensions from the duodenal glands (Brunner's), leaving the muscularis unbroken except where the ulceration has taken place. The rapidly growing neoplasm has led to softening with the consequent necrosis of the papilla, similar to the ulceration of the superficial structures in cancer of the breast. Even underneath the ulcerated mucosa, partially preserved cancerous masses similar to the proliferated Brunner's glands at the border, were found. I consider the case clearly one of primary cancer of the duodenal gland. There was a diffuse small celled infiltration of the connective tissue; while the cells immediately lining the alveoli were columnar. Below these infiltrating glands, numerous solid nests of epithelial cells were lying between the muscle bundles. The cells are no longer of the cylindrical character, but rather spheroidal or polymorphous, with large round nuclei. Within this area are alveoli in whose centre mucoid degeneration has set in leaving only the outer rim of cancerous cells. A few of the outermost lobules of the head of the pancreas showed some infiltration of the tumour, but clearly differentiated from the parenchyma of the organ. Secondary metastases were present in the lymph glands of the lesser omentum, as far up as the hilus of the liver, but no secondaries were found in the liver itself. The cells of the metastases were larger and more spheroidal than at the primary seat.



Case II.—Section of bile papilla showing proliferation beginning in the duodenal glands at site of ulceration.

Malignant growths are by no means rare in the intestinal tract. Of these, primary carcinoma decreases in frequency from the rectum upwards until the duodenum is reached, when it again increases in frequency as we approach the stomach. Just what percentage of primary tumours of the intestine is confined to the duodenum is difficult to say; the Vienna tables places it at about 1 per cent., the Berlin tables give a higher figure. Later statistics place it as high as 10 per cent. (Heulin's table).

Of primary cancer of the duodenum over 50 per cent. are confined to the second portion about the bile papilla. In 45 reported cases of primary disease in the duodenum in which the localization of the tumour was definitely given, I find that 25 per cent. had their origin in the first part of the duodenum, 63 per cent. in the second part, and 12 per cent. in the third part. Of those beginning in the second portion, 76 per cent. involved the bile papilla.

The bile papilla is a structure in which we may have cancer arising from five different sources, and, as in each, the epithelium is either columnar or cubical celled, the cells of the aberrant growths can only with difficulty be distinguished as belonging to this or that source. Rolleston describes carcinoma of the ampulla arising from four different sources, and, as the ampulla is an integral part of the papilla, malignant growths of the ampulla are identical with those considered under the papilla. Thus, in the ampulla, Rolleston describes cancer arising from (1) the common bile duct, (2) the pancreatic duct (Wirsung's), (3) the epithelial lining of the ampulla, and lastly, from (4) the mucosa of the duodenum immediately surrounding. To this must be added a fifth origin of epithelial tumours of the papilla, namely, those arising from the duodenal or Brunner's glands. They have all been called carcinoma of the ampulla of Vater, and rightly so, as the invading growth soon involves it in extension, giving rise to objective symptoms of a tumour in this region. It is only in the few cases in which death has resulted before marked invasion has taken place into the adjoining tissues that a definite decision regarding the exact epithelial site of origin can be arrived at.

Authentic cases of cancer primarily arising from the ampullary epithelium are few. Rolleston (1901) after excluding all doubtful cases, finds only seven in literature, which along with the first of the above cases gives us eight reported up to the present. It is interesting to note that Halsted operated successfully on one of the seven cases, though he admits having made the fatal mistake in removing a portion of the growth in situ for microscopic examination, to which he attributes the cause of secondaries occurring some months later.

That Brunner's glands may be involved in malignant growth without involving the epithelial lining, or crypts of the duodenum, I wish to emphasize, as is seen in the histological findings in the second case. In it, along with the anomaly in which the pancreatic duct and the bile duct open by separate orifices on the duodenal mucosa, existed a carcinoma of the papilla springing from Brunner's glands. The malignant growth was situated at the biliary orifice and can hardly be classed as a cancer of the ampulla. There was no ampulla, but the location of the tumour was that of a normally placed one. The course of the pancreatic duct, unassociated with the bile duct, removed all suspicion of extension from it or the pancreas. As it happened, in this case the Brunner's glands involved were those immediately surrounding the papilla and invasion took place into the deeper structures and about the bile duct. This invasion was sufficient through pressure to obstruct the duct, with the consequent jaundice and its complications. As the pancreatic duct was uninvolved there was no retention of pancreatic secretion and hence digestion was not markedly disturbed for some time. At autopsy the carcinoma had progressed to ulceration which destroyed the papilla.

The first of these cases was one of true carcinoma of the ampulla. It is indeed remarkable how small sized a tumour, springing from the lining of this recess, may lead to a fatal termination. This invasion, though not extensive macroscopically, was sufficient to cut off the lumen completely. Following the obstruction of the bile and pancreatic fluid the ducts in both the liver and pancreas were greatly dilated. In looking at the papilla from the duodenal surface the appearance was that described by Rolleston as seen in primary carcinoma of the ampulla. The mouth of the papilla was gaping and within it could be seen a small tumour mass. A vertical section through the papilla showed the bile and pancreatic ducts to be intact, and the neoplasm to exist below the duodenal mucosa and above the head of the pancreas.

In those cases in which the bile duct and parcreatic duct meet at some distance from the papillary orifice it can readily be seen that a cancer arising in the ampulla would be recognized without difficulty as having its origin in this region, but such a case has, to my knowledge, not been met with.

Regarding the symptoms worthy of confidence in the diagnosis of duodenal ulcer, Whittier places foremost those arising out of obstruction of the common bile duct, and the effects following the lessening in calibre of the intestine. Now, in the reported cases of carcinoma of the papilla, there are few in which the growth beginning in the papilla spread about the circumference of the bowel. Nearly all cases had a fatal termination before this stage was reached. The infiltrated papilla projects into the lumen of the intestine, and here bears the brunt of the irritation from the intestinal contents, with a diminished blood supply ulceration occurs early, with a consequence that blood may appear in the dejecta and vomitus. Obstruction of the bowel thus plays little part in the clinical history of cancer of the papilla.

Jaundice is the most constant feature, and when present is progressive.

With the obstruction of the bile the stools are clay-coloured and offensive, the gall bladder is distended and the liver enlarged and tender. The early symptoms of indigestion are chiefly referable to the absence of bile from the intestine. If the obstruction also involves the pancreatic duct, the indigestion is exaggerated and the pain and tenderness over the pancreas is much increased.

Vomiting is an early and fairly constant symptom, but presents variable characters. At the beginning, vomiting usually sets in a short time after meals and is due to an increased irritability of the duodenal mucosa. Later on it becomes more frequent, with no relation to food taken and showing complete absence of bile.

Pain is an uncertain and unreliable feature, being due to varying causes. The pain of ulceration, flatulence and occlusion of the bile duct may all be present at the same time and so be of no service in basing a diagnosis. During ulceration and obstruction of the papilla, periodical lancinating pains are present, not unlike those seen in biliary colic. Some even go so far as to say that a number of cases have their origin in the presence of an obstructed stone in the ampulla of Vater. No such close relationship between gall stones and cancer of the papilla exists as is found with malignant disease of the gall bladder.

The presence of a tumour is rarely discoverable on account of the position of the second portion of the duodenum. Moreover, as has been said, a tumour involving the papilla is usually so small that its presence, even in a favourable position, would readily escape detection. Should a tumour be felt, it is most likely to be mistaken for the head of the pancreas.

Emaciation is rapid and fever is seldom present.

It is the exception rather than the rule to find extensive metastasis, and it is seldom that other than a few lymph glands in the immediate neighbourhood become infiltrated. The chances for a septic cholangitis in the dilated bile ducts is great.

The duration of the majority of the cases on record is from five weeks to six months after the first symptoms appear, and up to the present, Halsted has been the only one to attempt a radical operation.

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ON THE PRESENCE OF SOAPS IN THE ORGANISM IN CERTAIN PATHOLOGICAL CONDITIONS (A PRELIMINARY COMMUNICATION).

By OSKAR KLOTZ (by invitation).

This I can quite confirm, that the centre of the calcareous deposits consists of calcium salts laid down in a homogeneous matrix (Litten), but this is not the case at the periphery, where the active process of calcification is taking place. On the contrary, here we find substances which chemically react as soaps, and can be isolated as such. Such soaps are here present as the potassium, sodium, or even the ammonium compounds of the fatty acids, being rendered insoluble by the combination with albumins of the degenerating tissues. Such soap-albumin compounds, in the presence of the calcium of the blood, form insoluble calcium curds or double calcium soaps. These insoluble calcium substances are then decomposed into the phosphate and carbonate of lime, which, as has been said, remain as the permanent deposits of calcium in a homogeneous matrix. It is further shown that soaps may be demonstrated in pus, particularly that of chronic abscesses, and Langerhaus has shown that a calcium soap is formed in fat necrosis.

In the production of an experimental calcareous degeneration in the kidneys of rabbits, it is shown by means of staining that the fatty changes taking place in the cells of the tubules is in the same location as the calcareous deposit, and further that in these kidneys a soap may be demonstrated by proper chemical analysis.

The following conclusions are arrived at:

1. The earliest change in cells, which later undergo calcareous degeneration, is one of cloudy swelling or coagulation necrosis.

2. Following this, fatty changes are noticeable in the cells, and now, by means of proper reagents, soaps with potassium, sodium, and presumably ammonium bases, can be detected.

3. Such soaps and albumins form a combination which is insoluble in water or salt solution.

4. Soaps and fatty acids have an affinity for the calcium salts in solution in the body-fluids, and form with them an insoluble compound.

5. Later, judging from the fact that phosphate and carbonate of lime are formed, and the deposits give no reaction for fats, the fatty acid moiety of the calcium soap is replaced by the more powerful carbonic and phosphoric acids.

SOME FALLACIES IN THE AGGLUTINATION TESTS;

A PLEA FOR THE EMPLOYMENT OF A MORE UNIFORM TECHNIC.

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The increased attention which has of late been extended to the serum agglutination of bacteria has brought into prominence certain fallacies which arise in the experimenter's hands, and which must have been noted, to a greater or less extent, by everyone working in this subject.

The literature which has now accumulated dealing with the agglutination of the typho-coli group alone is nigh unlimited, and many of the reports are so at variance with each other that we are landed in a hopeless confusion when we strive to draw the true inferences from them. Some have held that the agglutinins produced by each organism are specific, while a host of others have denied this and have pointed out that they are only special for closely allied groups of organisms. Others, again, have demonstrated that this agglutination reaction is not necessarily a vital one, but is also exhibited in inert substances in a fluid medium. This latter phenomenon, Sir Lauder Brunton[1] and Norris demonstrated by the use of bits of soaped cork floating on water which had been faintly acidulated. Hence, in recent years, one would almost be led to believe that the Gruber-Widal reaction, which was to become the only absolute test in differentiating various infections and to group the different organisms into their respective classes, must be overthrown.

Much of this confusion has been due to the different methods employed in the technic; in fact, there are almost as many methods as there are workers in the subject. In such delicate tests as are required for the agglutination reaction, our present methods, though much advanced through the experiences of eight years of research, are still very crude, so much so, in fact, that many personal errors, including technic, are only eliminated with difficulty. It was a similar state of confusion which led American bacteriologists to adopt a standard method of procedure in determining the biologic characters of bacteria.

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[1] Lauder Brunton: Jour. Path. and Bact., 1901, vol. vii, p. 53.

Quite recently, Nicolle[2] has emphasized the necessity of the various workers employing a uniform technic. It is the first essential if we wish to obtain some comparable reports and results of agglutinins, to adopt common methods, not only in the instruments we use, but also in the treatment of the animals and cultures we employ. The fact that one observer obtains agglutination of an organism, even in a stated dilution of his serum, neither tells us the susceptibility of his organism nor the potency of his serum; all depends on the age of the culture, the medium in which the agglutination has been carried on, the temperature of incubation, the reaction of the medium and many other points which will be discussed in turn.

AGE OF CULTURE.

The term "age of culture" may mean one of two things—either the length of time that a given culture has been growing in or on one particular mass of medium, or the time that the organism has been isolated from the animal body or from its natural habitat in water and transferred to artificial media. Here we refer to the latter.

That environment plays a great part in modifying the characters of an organism is well seen in the following: Rodet, and Nicolle and Trenel[3] found that the *B. typhosus*, isolated from typhoid patients, would agglutinate with great difficulty unless it had been retained on artificial media for some months. Bail[4] observed this same difficulty in agglutinating organisms isolated from water, but that this difficulty could be partially overcome by heating the organisms to 60 C. Wyatt Johnston[5] pointed out in 1897 that the power to agglutinate varied widely with different cultures of the same organism more especially, he said, with the degree in virulence. The relationship of agglutination to virulence seems to be only one of activity of the organism. Thus a strain which is frequently transferred to fresh media, and hence culturally becoming more active with increase in its virulence, is found to be more sensitive to agglutination.

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[2] Nicolle: Annal. de l'Institute Pasteur, 1904, vol. xviii, p. 209.

[3] Nicolle and Trenel: Annal. de l'Institute Pasteur, 1902, vol. xvi, p. 562.

[4] Archiv f. Hygiene, 1902, vol. xlii, p. 307.

I found the same difficulty as Rodet and Bail in agglutinating newly isolated strains of the *B. typhosus.* A laboratory stock culture of *B. typhosus* which had been on an agar medium for over ten months was grown in a flask containing 400 c.c. of water and 50 c.c. of neutral bouillon. Another strain of *B. typhosus* recently obtained from the spleen of a patient, was taken from the first transfer on agar and inoculated into a similar mixture of broth and water. Both strains, incubated at 37 C. for eighteen hours, were tested against an alien typhoid-immune serum (rabbit).

DILUTION OF TYPHOID IMMUNE SERUM.

	I-60	I-120	I-310	I-620	I-930	I-1240	I-2480
Old laboratory stock.	+ +	+ +	+ +	+ +	+ +	+ +	+
Recent strain.	+ +	+	±	0	0	0	0

In the table the following signs are used: + + to indicate strong agglutination. + to indicate fair, but not intense and rapid. ± to indicate partial.

Evidently strains of *B. typhosus* newly isolated from the animal body have a diminished affinity for agglutinins. It would seem that the agglutinin receptors of the micro-organisms are already satisfied or partially satisfied by the agglutinins obtained from the reaction of the tissues of the recent host. That an organism once agglutinated, whether *in vitro* or *in vivo,* retains the inability to unite with the agglutinin haptophores, is borne out in all the experiments; and hence in testing the potency of a serum we must be careful in selecting our test organism.

The growth of the old laboratory stock strain in the flask was quite different from that of the recently acquired strain. The former showed a medium equally clouded throughout; the latter, though the medium showed similar cloudiness, also showed a fine granular layer at the bottom of the flask which consisted of pseudo-clumps. These clumps, microscopically, did not differ from true clumping except that, in the medium about them, there were still actively motile organisms. The lower limit of the dilution, obtained in the strain recently isolated, has been our experience in all such tests; and this, too, is the case when the organism has been isolated from the blood or feces of a patient. Different strains of *B. typhosus* do not agglutinate uniformly until grown for at least six months on agar. Up to this time they increase in susceptibility to the agglutinins. This latter point opens up a question: Can we distinguish, by the avidity with which an organism, isolated from water, becomes agglutinated, how long that organism has dwelt outside the animal body? The clearing up of this point would give the absolute value of finding *B. coli communis* in drinking water as establishing its source from recent contamination.

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[5] Wyatt Johnston: Trans. Amer. Pub. Health Assn., 1897.

MEDIUM IN WHICH AGGLUTINATION IS CARRIED ON.

All living cells are influenced, more or less, by the medium in which they are grown; either by its affecting their morphologic characters or perverting their secretions. Now, the agglutination process depends on a chemical or at least on a physicochemical reaction. This reaction must, of necessity, vary according to the nature of the secretions. The agglutinable substance produced by the micro-organism must take the form of an excretion which, along with other products, is modified by the medium in which the micro-organism is grown. The whole question of whether or not a microbe agglutinates well with its homologous serum (granting that the serum is potent) depends on the quantity of agglutinable substance it has produced or can produce. This frequently depends on the method of culture employed. Some prefer growing the micro-organism in a plain neutral broth to which an active serum is then added. Others use specially prepared media, as in our own[6] case, where a weak broth is used so as to dilute the albumins and proteids in the medium as much as possible without affecting the growth of the organism. Our dilute broth was made up of 450 c.c. of water, to which 50 c.c. of beef broth (as described by the Committee of American Bacteriologists) was added. We thus obtained a uniformly cloudy medium which was always of the same opacity after eighteen hours' incubation. Others, again, prefer to grow the microbe on agar, from which an emulsion is then made in water or in sterile salt solution, equal to the opalescence of a given solution. This will be referred to later.

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[6] My remarks apply to the methods in use at McGill University, Montreal.

In 1901 Savage pointed out that a spontaneous clumping occurred in certain of his strains of *B. typhosus,* but always in a broth medium. The clumping appeared mostly in cultures several days old, but it was also seen in twenty-four-hour growths. No modification in a medium could be found which prevented its occurrence or modified its appearance. Such a spontaneous agglutination was never noted by us in using the dilute broth medium.

A year-old laboratory culture of *B. typhosus* was grown in our weak broth medium, and another transfer made from the same strain into an artificial broth medium as used in the laboratory, made from Liebig's meat extract, using 3 grams of the extract, 10 grams of Witte's peptone and 5 grams of salt to 1,000 c.c. of water. Both were under the same conditions of temperature, time and reaction. After eighteen hours' incubation, each was tested against a common immune serum. It was found that the clumping in the weak broth appeared sooner, but, at the end of two hours, the reaction in it was not so nearly marked as in the stronger broth used. In seeking an explanation it was found that the filtrate of the more concentrated medium gave a precipitate with the serum, while the weak medium did not react. This brings up another point—that of peptone precipitants present in artificially produced sera due to inoculating peptone cultures into the experimental animal. We know that various proteids inoculated into the animal body will call forth the reaction of an antibody, which antibody, in the presence of the substance injected will cause a precipitation. With the latter view of Wassermann, that agglutinins and precipitins are identical in character, this would mean that the agglutinable substance within the body of the organism will, under favorable conditions, be extruded into the surrounding medium, and will exist in it as free bodies ready to be acted on by the agglutinins. Now, in carrying out experiments regarding agglutination, it is of great import to concentrate the agglutinable substances within the body of the microbe and to have as little free in the medium as possible. That the free precipitating body in the medium is identical with the agglutinable substance, is not proven as yet, but true it is that frequent washing of an organism relieves it of the power to agglutinate when acted on by an active serum.

The reaction of the medium in which the microbe is grown also plays a part in the sensitiveness of the microbe to agglutinins. I have found that *B. typhosus* is most sensitive when grown on media neutral or faintly acid, +2.0 N/10 to phenolphthalein.

Further, Bordet[7] points out that agglutination takes place much more actively in emulsions of the organism made in normal salt solutions, than in emulsions made in sterile water without the presence of salt.

TEMPERATURE OF INCUBATION.

Cultures obtained from the same stock and inoculated into weak broth media, but placed at different temperatures of incubation, are found to show a different power of agglutination. *B. typhosus* will grow well at 37 and 42 C. This organism grown at these temperatures shows a more active agglutination reaction in the cultures grown at 37 C. At this temperature well-marked agglutination was obtained at a dilution of 1 in 4,960, while only a faint agglutination resulted with the same serum in a dilution of 1 in 2,480, in the colony grown at 42 C. Whether there are more or less products excreted by the organism at one temperature than at another, which have an influence on the agglutinative reaction, or whether there is a difference in the number of organisms present in the medium, or both, I am not prepared to say.

Under this same heading I would also note another interesting phenomenon in the technic of agglutination. If a culture of the dilute broth be taken directly from the incubator at 37 C. and placed in the cold, the typical agglutination flocculi will result. This can be demonstrated very prettily by growing the *B. typhosus* in long tubes, such as described by Adami and Chopin[8] for macroscopic agglutination tests. After incubating eighteen hours place the tube in ice water. Within twenty minutes very decided flakes will be seen adhering to the sides of the tube which, in the course of several hours, will be precipitated in flocculent masses. The carrying of these tubes from an incubator at 37 C. to a cool laboratory at 17 or 18 C., is often sufficient to chill the culture and produce the pseudo-clumping.

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[7] Bordet: Annal. de l'Institute Pasteur, 1899, vol. xiii, p. 225.

NUMBER OF ORGANISMS PRESENT IN TEST EMULSIONS.

In our present belief that an immune serum contains agglutinins which are limited in amount or number for such serum, we can conceive that there must be an equally limited number of receptors which will exactly neutralize that serum. It matters not whether we consider agglutination as a chemical or a physico-chemical reaction. Should these receptors be too few in number, there being too few organisms in the medium acted on by the serum, there will be a definite amount of agglutinin, which is still potent, unsatisfied. This is somewhat similar to the observations of Castellani, who found that, in sera containing several varieties of agglutinins, he could satisfy and remove one without affecting the other. Thus from a serum which agglutinated both the *B. typhosus* and *B. coli communis,* he could remove the agglutinins for either one by subjecting the serum to the respective organisms and still leaving the agglutinin for the other unaffected.

Again, if a medium contain so many organisms that the receptors are more numerous than the agglutinative haptophores of the serum, the total amount of the receptors will be unsatisfied by this serum with a consequent imperfect agglutination reaction. Thus it is impossible to compare the agglutinability of two strains of the same organism unless the condition as to the number of receptors—or, in other words, the number of bacteria—is the same. To obtain a medium having a standard number of organisms, Sacquépée and, later, Nicolle, used as indicators of opacity, suspensions of silver chlorid and lead carbonate, respectively, which were made with a definite formula. Emulsions of bacteria, either in broth or normal salt solution, were then made to the same degree of turbidity which represented the amount of opalescence in an eighteen-hour culture. We found that, by growing the organism in a large amount of medium—500 c.c. of weak broth—from a relatively small mass inoculated (three platinum loop fulls), that the degree of turbidity varied in imperceptible limits. When the food supply is in such relatively large quantities to the number of organisms inoculated in it, the growth, in the first eighteen hours, is constant.

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[8] Adami and Chopin: Jour. Med. Research, 1904, vol. ii, p. 469

A control emulsion of silver chlorid was prepared equal to the turbidity of one of the flasks, and this was compared with that of the succeeding cultures. When the time of incubation was kept constant, the opalescence of the growth did not vary in the different flasks.

MEDIUM USED IN INOCULATION OF ANIMALS.

Another process which leads to discrepancies in the agglutination test is the diluent used in the inoculation of animals for serum production. It has been frequently shown that the inoculation of proteids from different sources sets up in the animal the production of an anti-body in the form of a precipitin. Under these proteid bodies come also peptone and meat extracts. The injection of these substances calls forth precipitins special for each, and, if the sera from the inoculated animals are added to proteid solutions similar to those inoculated, we obtain the deposition of flocculent masses—the precipitate. Now, in inoculating animals with broth cultures, we set up a series of reactions— the formation of agglutinins for the microorganism and a special precipitin or precipitins for the proteid elements in the medium. The serum thus obtained, when tested against another broth culture, will show a distinct macroscopic reaction, whether the specific organism be present or no, the organism being mechanically carried down with the precipitate formed and appearing to afford in all respects a typical agglutination reaction.

To obviate the confusion arising from agglutinins and precipitins, the organism must be grown on solid media from which it is then removed with the platinum loop and emulsified in sterile water, or better, a normal salt solution. This emulsion, when inoculated, will produce no precipitins in the blood serum.

AGGLUTINATION REACTION DUE TO OTHER THAN SPECIAL SERA.

Agglutination following the addition of small dilute quantities of certain chemicals, has an interest beyond the mere experimental one. Such chemicals are used, not infrequently in disinfecting our reagent glasses, and also as preservatives for agglutinative sera. Among the reagents which, when added to a sensitive culture, produce the typical agglutination reaction, are mineral and organic acids, perchlorid of iron, calcium chlorid, mercury bichlorid, safranin, vesuvin and sodium sulphid; strong solutions of formalin will also cause the agglutination. Carbolic acid solution and chloroform are without effect, and hence can be safely used as antiseptics in preserving immune sera.

Blachstein,[9] working on the supposition that agglutination was a purely chemical phenomenon, concluded that there must be substances which will act equally specifically as the active sera against the homologous organism. Such a substance he found in chrysoidin which, in weak dilutions, has specific agglutinating reaction against the vibrio of cholera. Later it was shown that dilutions of chrysoidin would also agglutinate the vibrio of Finkler-Prior, though not in so high dilutions as was obtained with the cholera organism.

The dried blood test, which was instituted by Wyatt Johnston,[5] has its many advantages to the practitioner, but care must be taken in the selection of the paper on which the blood is collected. Testing a series of papers containing no blood, I found that the microscopic clumping could be obtained with some by using as an agglutinin a drop of water which has been in contact with the paper for some minutes. All papers do not act thus, but those which have an exceptionally glossy finish, as the cheap note paper, and leave a dull area where the water has been in contact, give a clumping of the organism.

Blotting paper, such as is used in the Talqvist hemoglobinometer, serves very well to collect suspected blood. and does not introduce any foreign clumping agent in the test. The blood stains are cut out and soaked in a watch glass with normal salt solution. The solution then represents our blood serum to be used.

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[9] Blachstein: Contribution to Science, Johns Hopkins Univ., 1900, p. 891.

Wesbrook[10] has devised a method which Wilson has put into practical use for making accurate dilutions of dried blood specimens, which the ordinary blood collection on paper does not allow. He provides the physician with a small sheet of aluminum foil on which the blood drops of a suspected typhoid patient are collected and allowed to dry. In this way the blood of a suspected typhoid case is sent to the laboratory where the dried blood is carefully shelled off of the foil and weighed. From numerous experiments he found that 0.001 gm. of the dried blood was equal to 0.004 gm. of the fresh blood. The weighed dried blood is then mixed with a sufficient amount of sterile normal salt solution to make a dilution of one in fifty or more. As Wesbrook has pointed out, the main difficulty in using this method is the weighing of the blood specimen. A little practice obviates this difficulty.

MACROSCOPIC VS. MICROSCOPIC METHOD OF EXAMINATION

Each method has its advantages and disadvantages, and each observer has his own reasons for choosing the one or the other. In testing strains of organisms against different sera, we have, at this laboratory, given preference to the macroscopic technic. By carrying out the technic recently described by Adami and Chopin, the observer is relieved, to a great extent, of the fatigue in using the microscope, and, further, he can compare all his dilutions at a glance, gaining a much more exact picture of the potency of the serum and the sensitiveness of the organism. Much finer grades of dilution can be arrived at by using these large quantities of bacterial emulsion than is possible, even with the use of the mixing pipette of the hemocytometer, in the hanging-drop preparation.

SUMMARY.

Without entering into the discussion as to the chemical or physico-chemical nature of agglutination and the bearing this has on the differences in the agglutination reaction, and vice versa, I wish to point out that in carrying out these tests we require a greater uniformity in the technic to allow comparisons of the results obtained by different observers.

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[10] Wesbrook and Wilson: Philadelphia Med. Jour., 1898, vol. i, p. 549.

The susceptibility of an organism to agglutinins increases for the first six months from the time it has been isolated from the animal body and grown on artificial media.

Inoculations of animals with broth culture will produce in their sera, beside the agglutinins, precipitins whose reaction in broth culture can not be distinguished from true agglutination, as the organisms are mechanically deposited in the precipitate.

Pseudo-clumping may be obtained by using emulsions of bacteria in undiluted broth and testing against the above serum.

The agglutination of a micro-organism varies with the medium in which it is grown, the reaction of that medium, the temperature of incubation, and the number of organisms present in the emulsion.

Pseudo-clumping may be produced by sudden changes in the temperature and by the addition of certain chemicals. The addition of carbolic acid or of chloroform as preservatives in an immune serum does not interfere with the agglutination reaction.

In using the dried blood test, paper having a soluble gloss should be avoided for collecting the blood.

In our hands the macroscopic method for determining agglutination has proved the most useful and rapid. A time limit of three hours has been placed on all these reactions.

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STUDIES UPON CALCAREOUS DEGENERATION.[1]

I. THE PROCESS OF PATHOLOGICAL CALCIFICATION.

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PLATES XXX AND XXXI.

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INTRODUCTION.

It is a well known fact and a not uncommon occurrence that the interest in certain subjects or lines of thought fluctuates widely; we are ever too ready, when we think we have obtained an explanation of a certain phenomenon, to take much for granted and allow our enthusiasm to lapse. Such has been the case in the study of pathological calcification, which has gone through several periods of new theories and rest.

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[1] This study was begun while the writer was Governors' Fellow in Pathology of McGill University, and has been continued and completed under a grant from the Rockefeller Institute for Medical Research.

Notwithstanding these theories, in reviewing the literature concerning calcareous degeneration or calcification, one is struck with the dearth of actual facts that are set forth upon which those theories are based. In truth the labor that has been spent in the actual study of the *process* has not been extensive and it is confined to a few papers in the last decade. There have been many who have reported the occurrence of calcareous degeneration of diverse tissues and have even gone minutely into the chemical nature of the organic salts present, but here the study has ended. Regarding the existence of pathological calcification we have abundant data, as to the process of calcification we know little.

Indeed, calcification is one of the commonest pathological conditions noted by the older writers, especially that occurring in the arteries and in fibromata of the uterus. Not infrequently the change in the arteries is spoken of as appearing "partly fatty and partly earthy." No doubt reference was made to the yellow atheromatous areas overlying calcareous plaques in the arterial wall. Not until Virchow in 1858 asserted that inorganic salts were deposited in supersaturated solutions of the same and that the deposit of calcium was of this nature, was there any discussion on the subject. Virchow held that calcareous deposits were chiefly found in old age and in diseases causing wasting of the bony framework, and that in this state calcium was dissolved out of the osseous system and on account of the too concentrated solution of calcium salts in the blood these were laid down elsewhere as metastatic deposits. In this way he explained the common occurrence in old people of calcareous plaques in the arteries.

Some years later von Recklinghausen reported the studies he had made upon the degenerations. He pointed out that calcium deposits result partly in tissues which are well supplied with tissue fluid, but also in tissues poorly supplied. When blood or tissue fluids are saturated with calcium salts, due to repeated breaking down of bony structures, he noted a secondary incrustation of the lung and kidney tissue. But there were other cases in which, as in fibroids and phleboliths, calcification proceeded under conditions of normal calcium content of the blood. Von Recklinghausen looked on the processes of ossification and petrification as similar chemically, though differing biologically, in that the former took place in active and living cells, the latter in degenerating or dead tissues. He held that the factors governing calcification were: (i) the separation of sparingly soluble lime salts, (ii) the existence of these substances in excess in the fluids, and (iii) the presence of a foreign body which acts as a nucleus.

These factors would be of weight in considering fluids outside the body but not within. The lime salts in the tissue fluids are not found in excess during calcification and the common salts of lime found in the tissue fluids are very soluble. The deposit of calcareous plaques in the serous membranes and in the arterial walls he considered under ossification and as distinct from calcification.

Lancereaux's views were very similar to those of von Recklinghausen, though he attributed the supersaturation of lime salts in the blood to a hindrance of the excretion of these salts by the urine, and found them accumulated in the pyramids of the kidney and the mucosa of the stomach and intestines.

Thus it was that up to the time of Litten's ingenious experiments (1881), the theory of the excess of lime salts in the blood was accepted as explaining calcareous degeneration. No note was taken of the predilection of certain tissues for this deposit of lime, except that dead or dying tissues were regarded as a nucleus about which the carbonate and phosphate of calcium would crystallize. With Litten's studies—which will be discussed more fully later—a new theory was raised which superseded that inaugurated by Virchow, and up to the present time his has been the explanation of calcification accepted in the textbooks. After the appearance of Litten's work many followed in his footsteps, the majority supporting his views, although some have disagreed with him. The prevalent view, based on that of Litten, is briefly (dealing particularly with the cases of calcareous degeneration in the kidneys) that one or more irritants act on certain tubules in the kidney through which these irritants are excreted, and during their passage through the kidney cells, so injure the tubular epithelium that a degeneration of the cell protoplasm occurs, and that this degenerated cell protoplasm has an affinity for, and fixes as an insoluble compound, the calcium salts brought to it by the lymph and blood. These, it is maintained, are the essentials for the deposit of calcium salts, and it is pointed out especially that a coagulation necrosis of the renal epithelium is a necessary stage in the process.

This view has been generally accepted; and yet it is noteworthy that no one has proved that outside the body there is to be obtained the formation, even temporarily, of compounds of lime and any form of proteid. The present investigation was undertaken, in the first place, to determine with what organic substance present in degenerating tissues it could be shown that lime salts entered into combination. It was in repeating Litten's and von Kossa's experiments and in studying also the changes occurring within celloidin capsules containing various substances, and introduced into the peritoneal cavity, that indications were gained leading to the results about to be detailed. It was first undertaken to produce, experimentally, calcification of the kidney and other tissues and to study the successive changes to be noted in these. The rabbit was most frequently used in these experiments, partly on account of the normally high calcium content of the blood, and partly because these animals stand various operations very well. But in addition many calcified tissues from the human body were studied, the process in connection with arterio-sclerosis especially, and, as will be detailed, not a few experiments of other nature were made during the course of the investigation.

In studying the calcareous degenerations, the tissues were subjected to a series of tests as to the staining qualities and the chemical nature of the material. From all the experimental work on animals and the analyses of human degenerating tissues, one conclusion was always arrived at, namely, that calcareous degeneration is preceded or accompanied by deposits of a soapy material. This soap exists in chronically inflamed tissues in advance of the deposit of lime, or, if the lime is already to be found in the tissues, the soap appears in the peripheral zone of the calcareous infiltration. Moreover, it would seem that with the first deposit of soap, calcium salts are immediately attracted to it from the body fluids. Such progressive calcareous deposits are marked by the appearance in the periphery of groups of granules, some of which are found to react for both calcium and soap, others for soap only,—that is, some of the soap deposit has been converted into a compound containing calcium; in other words, calcium exists in the recently calcified areas as calcium soap.

That this calcium soap does not continue to exist as such was noted in old calcified fibroids and other tumors. In these it was seen that, except in the advancing border of calcification, the dense calcareous deposits are composed chiefly of calcium carbonate and calcium phosphate with no soap in its substance. It would seem that the fatty acid moiety of the calcium soap had here been replaced by the stronger phosphoric and carbonic acids to form the more stable compounds.

This is, in brief, the trend of the paper.

Before proceeding with the description of the work I wish to extend to Professor Adami my best thanks for the supervision of the entire work and for his unfailing help and the many suggestions which have been of great aid to me during the course of this and other researches.

STAINING METHODS.

Calcium and Calcium Compounds.—As a routine all specimens were stained with hematoxylin and eosin. These stained specimens gave the general character of the section, and, moreover, the presence of calcium salts, when in fair amounts, was indicated by the appearance of fine blue-black dust-like granules, which stained darker than other portions of the tissue and were easily recognized. The hematoxylin will, however, only stain calcified areas when calcium salts are present in very fair quantities; slight amounts are unrecognizable by this stain.

To demonstrate calcium salts in tissues we have two other methods, one of which was of the highest service in my hands.

If tissues containing lime salts be treated for five minutes with a solution of pyrogallic acid (1 to 40 in water), to which one per cent. of sodium hydrate has been added, the lime salts will be stained a dark seal-brown while the remaining tissue appears a light-yellow color. Except in tissues where large quantities of lime salts are present, the stain is unsatisfactory, just as in many cases which show the presence of calcium salts with the following method the pyrogallic acid treatment gave no results. The most satisfactory method we have used is that recommended by von Kossa, namely, the treatment of sections with a 5 per cent. aqueous solution of silver nitrate. This solution picks out the most minute quantities of calcium by depositing an intense black precipitate in its place. The sections were immersed in the silver solution for from 3 to 12 hours, then washed in distilled water, and mounted in the usual way in Canada balsam. As the silver treatment of sections consists of an impregnation and precipitate in the tissues, and not a stain, we obtain a much exaggerated picture of the actual quantity of lime salts present. However, this is of all the more service when minute quantities of earthy salts exist, and the method is not proposed as affording a quantitative measure.

As was pointed out by von Kossa, the blackening produced by the treatment of sections with silver nitrate is due to the production of silver phosphate which blackens in the presence of light; hence it would seem that a precipitation of silver is only obtained in the presence of a phosphate. The calcium deposit in the tissues exists, however, both as a carbonate and a phosphate, principally the latter, and this experiment would only lead us to detect the insoluble calcium phosphate. However, when sections containing calcium carbonate are treated for many hours with silver nitrate a certain portion of the two salts interact so that the calcium-carbonate granules become coated with a deposit of silver carbonate which in sunlight gives off carbon dioxide leaving the black silver oxide, or we can hasten the stage of blackening when some silver carbonate has formed by first thoroughly washing the specimen in distilled water and then treating it with a dilute soluble sulphide.

There is, however, I would point out, a means whereby the calcium existing in any favorable combination in the tissues can be demonstrated by the silver-nitrate method; and this consists in first converting calcium salts into the chromate by means of potassium chromate and then replacing the calcium by the silver, thus obtaining the silver chromate which blackens on exposure to sunlight. Under ordinary conditions the calcium chromate is soluble in water, but when tissues containing calcium are acted on in the cold we find that the calcium chromate does not leave them, and on further treating them with silver nitrate the red silver chromate is seen to become deposited in their sites. The use of silver nitrate in these impregnation tests is we have found facilitated by acidulating it with acetic acid. This same method demonstrates calcium when combined with fatty acids to form soaps.

I would point out that these micro-chemical tests for calcium do not demonstrate the actual calcium salts, but by replacement we demonstrate their presence and location with blackened silver salts.

Virchow and Halva have both analyzed calcareous nodules by chemically demonstrating the presence of both phosphate and carbonate of lime. However, they point out that the phosphate is much in excess; while Chiari found only phosphatic salts in the calcified nodules examined by him.

The method of staining lime salts with silver nitrate gave beautiful pictures in all of the tissues examined. Especially was this true of the sections of experimental calcification of the renal tubules (vide Fig. 2, Plate xxx.). It is true that the metals, mercury, copper, and lead, will give a black precipitate in tissues with silver nitrate, but they can be excluded in these cases when other evidences are given of the identity of the calcium.

However, in the experimental production of calcium deposits in the kidney we have to deal with another factor not often met with in the slowly forming calcareous nodules. In the former we are inducing a sudden change by means of chemical or mechanical reagents, and this has an effect on the general metabolism. Especially is this so in the inoculation of animals with such poisons as corrosive sublimate, lead acetate, or copper sulphate. Here besides acting directly on the kidney tissue we produce an hæmolysis of the red blood cells and thus occasionally, depending on the strength of the inoculation, we find an iron deposit in the same convoluted tubules, which are the seat of calcareous degeneration. Similar though less amounts of iron can be demonstrated in the liver; but that the presence of iron in the kidney is not the cause of the silver deposit, is to be ascertained from the fact that no calcium reaction results in the liver. The iron in both instances is detected by the Prussian-blue test. Attention has already been called by Gierke to the occasional presence of iron in specimens of pathological calcification.

In the routine examination of aortas we were struck by the frequency of stainable calcium salts found in either the media or intima where no lesion was suspected from the macroscopic or ordinary microscopic examination.

Fats and Fatty Compounds.—For the detection of fats, fatty acids, and soaps, I found that Sudan III gave the most satisfactory results. I have also used Scharlach R., which is a very closely allied stain to Sudan III, but found a difficulty in the employment of it as the stain precipitates very readily in its crystalline form into the tissues. Scharlach R. is the more intense but cannot be used in saturated solutions. Osmic acid impregnation of tissues to demonstrate fats is of more limited use, as we know that only the oleic acid and olein are blackened by it, while stearic and palmitic acids with their respective fats are left untouched. Hence in this work, outside of making comparisons of the quantities of olein or oleic acid and the total quantities of fats and fatty acids, sections were stained with Sudan III; osmic acid was used only very occasionally. The nature of Sudan III and Scharlach R. is such that they are insoluble in water but are soluble in alcohol, chloroform, ether, fats, fatty acids, and soaps. A saturated solution of the stain is readily obtained in 70 per cent. alcohol, and as the fats, fatty acids, and soaps are more ready solvents of it than is 70 per cent. alcohol, fat in sections treated with a saturated solution of Sudan III will pick out the stain while the rest of the tissue remains untouched. Our sections were usually stained 12–24 hours in the cold. (Vide Fig. 1, Plate xxx.)

Hence in Sudan III we have a stain which will differentiate fats, fatty acids, and soaps from other products in tissues. That soaps are ready solvents for Sudan III is readily demonstrated by making a concentrated watery solution of a soap and then adding the powdered Sudan III. The resultant mixture is a clear, deep red solution which can be filtered and further diluted. Such a stained though highly dilute solution, when evaporated on a slide gives a pinkish-yellow color, which is distinct from the golden-red staining of neutral fat. Fat particles even when emulsified to fine droplets retain this intense golden red appearance. The difference between the Sudan III fat stain and Sudan III soap stain is quite readily recognizable so that the two can be detected in tissues. It is more difficult to distinguish the neutral fats from the fatty acids in stained smears under the microscope, though olein appears distributed over the slide as stained oil globules which are easily picked out. These latter can be further differentiated by treatment with osmic acid.

It is to be noted that fatty acids as such are infrequent in the tissues, except in the case of dead tissues, such as gangrenous areas and pus, or as in the process of splitting up of fats in what are termed fat necroses. It is obvious that living cells do not pick up fatty acids unless, according to Fischler, it be leucocytes, in which case we can at times demonstrate fatty particles in the cytoplasm and show the vacuoles about the particles to be of acid reaction.

Fischler is most emphatic in his assertion that Sudan III is soluble in soap solutions, and the color produced on solution distinguishes this substance from the fat when stained in the same way, or, in other words, that fat and soap granules are to be differentiated in stained tissues. My results wholly confirm these statements. He states, further, that soap, when stained with Sudan III and then treated with 70 per cent. alcohol, immediately loses its color, the dye being taken up by the alcohol. This I failed to confirm wholly.

The statement is true of soap preparations in the test-tube, but, as we shall see later, soaps present in the tissues do not dissolve so readily in weak alcohols; hence they retain the stain after this treatment. As will be pointed out, these intracellular soaps do not exist as a free product, but form combinations with albumin with coincident alterations in reaction.

Calcium soaps are also stained by Sudan III, giving color reactions similar to those of potassium and sodium soaps. Calcium soaps, appearing in the tissues as a new deposit in which there is no excess of free calcium salts, are not distinguishable from other soaps. But when a portion of the calcium is freed and deposited either as carbonate or phosphate, we find, among the Sudan-III-stained soap granules, other, crystalline, bodies, highly refractile and not absorbing the stain. The latter granules, it is found, will precipitate with silver-nitrate solutions. In tissues therefore it is possible to treat the sections first with Sudan III, and later with silver nitrate, demonstrating the presence of soap granules and also showing amongst these free calcium salts not associated with fats.

Some might be sceptical regarding the ability to distinguish the finest fat particles from the granules of soap stained in sections. Such scepticism we can override by another method. If the material be cut by the freezing microtome, the sections quickly dehydrated in 70 per cent. alcohol, then washed with petroleum ether, the tissue may be rid of all the microscopic fat. If now soaps be present, the soap reaction with Sudan III will still persist. I do not wish to say that the ultimate fat is removed from the tissue, but all the stainable fat is taken out, and the petroleum ether has been without action on the soaps. Rosenfeld and Dormeyer have pointed out that tissues which do not show fat microscopically, when washed with ether or petroleum ether, still contain a considerable quantity of fat present within the cells; but the combined fats of this order give no reaction with Sudan III; they may be neglected in this regard. Here it may be noted that even after decalcified tissues have been prepared in the usual way for cutting in paraffin, by dehydrating in absolute alcohol and clearing with xylol, sections will still stain faintly with Sudan III in the regions of active calcification. I cannot but believe that in such cases we have to deal with soaps rendered less soluble by their combination with albumins (vide p. 659).

CHEMICAL METHODS.

The Determination of Soaps.—In our first experiments the method of Hoppe-Seyler was employed. The tissue under examination was finely ground up and extracted with boiling absolute alcohol. From the filtrate, evaporated to dryness, fats were later removed by ether or petroleum ether, and the residue was demonstrated to contain sodium and potassium soaps, along with small quantities of calcium soap. The residue on treatment with mineral acids yielded stainable fatty acids, all dissolving in water; the sodium and potassium soaps were precipitated with calcium chloride. As calcium soaps are so slightly soluble in alcohol, only small quantities of them were thus obtained and we had to rely on the flame test to demonstrate the calcium present.

The use by this method of hot alcohol for the extraction leaves it possible, perhaps, that a small amount of neutral fat may be converted into soap by means of the alkalies present in the tissues. As I was, however, dealing with calcified tissues, such as fibromata of the uterus, from which all the surrounding superfluous tissue had been removed and which were then washed with distilled water, I can hardly believe that sufficient sodium or potassium remained in the tissue to give rise to the formation of soluble soaps.

To obviate this possibility of error, the method was given up and the fats were extracted without the use of heat. After the material had been finely chopped it was dried over sulphuric acid and then extracted with petroleum ether in many relays, the powdering of the tissue being undertaken between each treatment with fresh petroleum ether. Dormeyer is very emphatic in his assertions that this method of treating tissues with ether, even when carried on for a long time, does not remove the last trace of neutral fats contained within the cell membrane. He prefers to digest with pepsin and 0.5 per cent. hydrochloric-acid solution, and thus break up all the cell bodies, whereby he claims to obtain 8.5 per cent. more of fat.

As the analyses here undertaken were not of a quantitative but only of a qualitative nature, these minute estimations are of no bearing. However, the gross fats being once removed, the powdered residue was treated with hot acid alcohol to dissolve out the soaps (which had been unaffected by the petroleum ether), and a filtrate obtained. From the filtrate the soaps were precipitated with acid calcium-phosphate solution, filtered off, and washed with ether. In tissues actively undergoing calcification, this residue was obtained in fair quantity—in fact, in greater quantity than in densely calcified tumors in which the amount of the calcium salt was altogether out of proportion to the fatty acid. The alcoholic extract of the residue, after treatment with ether and petroleum ether, yielded small quantities of calcium soap, which we must remember is only very slightly soluble in absolute alcohol.

THE PROCESS OF DEPOSIT OF CALCIUM SALTS IN BONE.

In Normal Bone.—In the above description of the methods employed I have had to refer to the results obtained, and, if only in a general way, I have indicated that, in tissues undergoing calcification, fats, soaps, and insoluble calcium salts are found associated in varying proportions, according to the stage of the process. We will now turn to a more detailed study of the process, and first consider whether under normal conditions—when calcium salts are laid down in bone—there is to be made out this same association; whether, in short, ossification and pathological calcification are processes of the same order.

The phalangeal bone of an infant six days old was examined by sectioning on the freezing microtome and staining with Sudan III. The head of the bone was cartilaginous with a centre of ossification within it. The sections were allowed to stain for eighteen hours, but at the end of this time did not take the Sudan III stain in any part. Both the cancellous and the compact bone were devoid of anything resembling fats, fatty acids, or soaps. The femur of a fœtal guinea-pig was next sectioned and treated in the same way and with like results; that is, we found that in normal growing bone there appears to be no stage in which fatty substances play any part in the deposition of the lime salts, and we are led to believe that this laying down of calcium salts in the bone is a secretory process on the part of the osteoblasts. Again, in sections of decalcified skull bone we found the matrix entirely devoid of microscopical fatty substance. The bone corpuscles, or lacunæ, remained unstained with Sudan III; nevertheless there was some neutral fat lining the Haversian canals and surrounding the contained blood-vessels. Thus we conclude that *the process of calcification in normal growing bone is a distinct process in which the specific cells of the part play the chief rôle in laying down the calcium salts.* There was nothing in the stained tissue which would lead us to believe that there is any connection between fatty substances and the advancing bone-formation at the epiphyseal line.

Pathological Bone Formation.—What is true with reference to normal bone was found to obtain also in new bone developed under pathological conditions. For this purpose we examined a small bony nodule developing in the tissues to the inner side of the lower end of the humerus, secondary to trauma and due apparently, as pointed out by Dr. Archibald, to a local separation of portion of the periosteum. In the second place, we examined a portion of a large nodular, pure osteoma of the left frontal bone, obtained from a man twenty years old, which had grown into the cranial cavity and had produced great atrophy of the frontal lobe. [Royal Victoria Hospital—P. M. Reports, No. $^{15}/_{05}$.] In neither of these specimens did Sudan III give any trace of the presence of fat or soap in connection with the more recently growing portions.

THE PROCESS OF DEPOSIT OF CALCAREOUS SALTS UNDER PATHOLOGICAL CONDITIONS.

In the Arterial Wall.—We now pass on to tissues which, under pathological conditions, undergo *calcareous degeneration.* Of these first and foremost are the arteries. All specimens examined by us, coming from persons over twenty-five years of age, showed more or less change in the aortic wall. The change varies from the faint white streaks which are seen through the intima, to the condition in which calcareous plaques are present, with and without ulceration.

The most remarkable point that strikes one in staining a series of aortas by the methods described, is the frequent presence of recognizable amounts of calcium salts in the media of adults. (Vide Figs. 3, 4 and 5.) In the routine examination of eleven specimens of the aorta, from persons varying in age from twenty-two to forty years, which on macroscopical examination showed no lesion other than fatty plaques in the intima, it was found that calcium salts were present in abundance at one time in the media, at others in the intima. It was not infrequently seen that the media was affected by the calcium increase while the intima remained unchanged or was affected by a slight extension only of the process from the media. We hope to call fuller attention to this change in the media in a subsequent article. So far it would seem to have escaped notice.

The white plaques in the intima have long been noted by pathologists and have passed under a host of names. Gross, as early as 1845, spoke of the condition as "atheromatous" and "steatomatous," where he says the microscope "brings into view a multitude of crystalline plates and fatty granules with albuminous and oily particles." Coplin has collected cases in which the change in the arterial wall was noted as early as the second year of life, but it is not our intention to deal here with the frequency of this affection. What is to be noted is that all vessels with arteriosclerosis have stainable calcium salts in either intima or media to a greater or less extent.

In studying normal aortas, taken from young adults, we found that neither intima nor media was affected by Sudan III, while only a few fat cells were seen in the adventitia. That is, in a normal and healthy state the intima and media of the aorta show no "microscopic" fat, and, further, when such aortas are stained with silver-nitrate solution there is no staining or deposit of silver in the tissues. Obviously the small amount of calcium salts and fat normally present in the tissues is not detected by our staining methods.

Sections were made from the iliac artery of an adult aged thirty years, which was apparently healthy on gross inspection, with the exception that here and there white areas of the size of a pinhead and not raised above the surface were present in the wall. The specimen was one which had been picked from a number as a sample of an apparently normal large vessel of adult life. When stained with hæmatoxylin, it was seen that the elastic layer did not follow the lumen of the vessel at an equal distance around the artery, but at the sites of the macroscopic fatty areas the intima was thickened towards and bulged into the media, and here the elastica was distorted and at times broken. Away from these isolated changes in the intima, the artery was found to be perfectly healthy. When stained with Sudan III, it was found that these thickenings showed the presence of numerous fatty granules, many of which stained an intense reddish-yellow. This fatty material lay in aggregated clusters of granular particles of larger and smaller size, situated chiefly at the border-zone of the intima, next to the media. While these fatty particles were seen in the nodular portion of the intima, the rest of the arterial wall was free.

In the healthy portion of the arterial wall the elastic lamina did not stain with Sudan III, and followed the lumen of the vessel at an equal distance and in a single strand. As we approached the intimal lesion, the elastic layer split longitudinally into a number of strands, which now, instead of retaining a regular wavy contour, became of unequal undulations and spread apparently into the media. The individual strands of the elastic lamina were swollen and fragmented, showing in places "a snapping" of the fibres. In this area of change in the elastica, the fibres were also seen to take the Sudan III stain, which became more intense and more granular as we approached the thickening of the intima. Immediately below the fatty deposits in the thickened intima, the elastic fibres were found much split up and composed of short, curly, Sudan-III-staining, strands.

When we come to examine closely the particles stained by Sudan III, we find them of different characters. First, we note the larger droplets or granules which are not perfectly round and which are isolated, or else we see several of these fusing into larger masses. The color of these is unmistakably that of neutral fats. Besides these we find many more smaller particles which are distinctly semi-crystalline and of a much lighter color, approaching almost to pink. These pink-staining particles are found in greatest abundance along the border-zone between the intima and media, and often occupying the elastic lamina itself.

It was found that when consecutive sections were stained alternately with Sudan III and silver nitrate, the silver nitrate was precipitated in areas identical with those picked out by Sudan III, though the relative quantities were unequal, there being proportionately more fat present. That is, the calcium deposit picked out by the silver nitrate occupies the nodular portion of the intima and is not seen in the rest of either intima or media. Further, the portion of the thickened intima picked out is that occupied by the pink-staining granules. In the less affected parts the calcium appears as fine dust-like material staining an intense black. Judged by the outline, seen as a silhouette against the lighter background, the individual granules here are crystalline. In places the elastic lamina was picked out as a wavy line composed of aggregated granules of calcium.

In this specimen we have the early change in the aorta, a condition which we would not yet speak of as arteriosclerosis. The white pin-head patches on the intima are seen to represent areas with fatty substance of two kinds: one an intensely yellowish-red staining material, which is fat; and lighter particles, which from their reaction with Sudan III can only be soaps; and, further, the thickenings in the intima are shown to contain an increase in the calcium content, and this increase to occur in conjunction with the light, soapy staining material. In other words, the calcium increases side by side with the soap; and the arrangement described suggests strongly that we are dealing with a deposit of calcium soap.

In another instance there was a generalized thickening of the arteries, though only at the bifurcation of the aorta was any macroscopical calcareous degeneration to be seen. The aorta showed numerous white plaques raised above the surface, and one of these from the abdominal aorta was sectioned. Stained with Sudan III both media and intima showed numerous aggregations of stained particles; the staining in the intima was confined chiefly to the atheromatous thickening in which considerable fat-staining masses occurred, while outside the nodule the granules were collected mainly round the elastic lamina. As in the previous case the granules could be divided into two classes: the larger masses which fused with other contiguous particles, readily recognized as fat deposits; and the smaller, lighter-staining granules which appeared semi-crystalline in shape. In the intima the larger fat masses were in excess of the finer granulations, while in the media the reverse condition existed; that is, the light-staining granules were much in excess of the fat, in fact only a little fat was to be seen on the boundary between the media and intima. In the rest of the media there were clusters of fine sand-like particles lying between the elastic layers and forming rows, more or less wedge-shaped, which occupied the position of the muscular fibres. In the media the stained granules were intermingled with numerous unstained refractile bodies, which disappeared from the specimens when treated with acid solutions. On treating the sections with silver nitrate it was found that the blackened deposit was present in the media in the regions where the Sudan III demonstrated the light-staining soapy particles. Similar to the Sudan III granules, the silver-nitrate deposit was aggregated into clusters lying parallel to and between the elastic fibres. The silver-stained particles in these clusters were much in excess of the refractile bodies seen in the Sudan III specimens; they tallied in number with those seen in unstained sections. The distribution of the silver-stained calcium salts was fairly general through the media, the greatest amount being towards the internal surface. (Vide Figs. 3 and 4.)

In the intima, however, there was less calcium to be seen, that present being confined to the atheromatous areas where the deposit seemed to be invading from the media. Beyond the atheromatous plaques, there was no lime salt to be seen in the intima excepting a fine, dust-like infiltration along the boundary zone. The whole condition in the arterial wall we look on as consisting of two distinct processes: the change in the media of a chronic nature and of longer standing than the intimal change, the degenerative process with fatty change having been progressive; and the atheromatous condition in the intima of more recent date, owing to which time had not been given for such extensive deposits of calcium salt.

We now undertook to remove all the neutral fats and fatty acids from the sections of the aortas above described by treating them with ether and petroleum ether. They were first washed in 70 per cent. alcohol before subjecting them to the ether. After this treatment the only material remaining which could stain with the Sudan III would be a soap. On examining the sections stained with Sudan III, it was found that all the bright-red staining neutral material had been removed and all that remained to take the stain were small crystals of a light salmon-red color. These crystals lie in clusters of 50 or more, the long axis of the clusters being parallel to the direction of the fibres. The clusters are to be found mostly in the intima at the atheromatous area and in this tissue lie some little way inwards from the endothelium. In the deeper parts of the intima the clusters become more broken up, and after staining with silver nitrate are seen to be mingled with calcium deposits, which are absent in the more superficial parts of the intima. Hence the Sudan-III-staining particles which remained near the boundary zone between the media and intima after treatment with petroleum ether are combined with calcium salts, while those, apparently of more recent origin, lying near the endothelial surface contain no calcium salt. When we examine the aorta beyond the atheromatous patch, we lose any evidence of soap in the intima except in the deeper portions near the media, and only as the atheromatous area is approached do the layers between the fibrous strands become widened and the spaces become the site of deposit of soap granules. In the media, where, as we have described, the calcium salts are much more marked, similar clusters of granules to those noted in the intima are found, with, however, the difference that in the media the clusters are compounds mainly of silver-nitrate salts—much calcium with a little soap,—while in the intima the proportion is exactly reversed.

To resume, it may be stated that by employing suitable methods it is found that calcareous deposits occur in the arterial walls, more particularly in the wall of the aorta, at an earlier age than is usually suspected.

These deposits vary as regards the region first affected. This variation may be in regard (i) to the internal elastic lamina, (ii) to the media, more particularly its muscle fibres, and (iii) to the intima in areas of nodose sclerosis.

Judging from the treatment of sections for the demonstration of fats and calcareous salts, the stages in this process are (i) degeneration of the affected region with deposit of fatty globules, (ii) formation of soaps, mainly with calcium, (iii) deposit of calcium salt devoid of a fatty moiety.

The results obtained in connection with calcified deposits in the arterial walls were confirmed and extended by an examination of other conditions of calcareous infiltration.

Calcareous Infiltration of Thickened Pleura.—The cases examined were evidently of some duration. There were thickened fibroid adhesions between the visceral and the parietal pleuræ, and in the centre of the fibroid layers dense calcified plaques occurred. The plaques were found to form rather irregular laminæ with somewhat irregular surfaces. By examining the material after decalcification, the section through what was a densely decalcified plaque was found to be stained a diffuse pale-yellow with Sudan III, no individual granules being present; but in the immediate neighborhood of the plaque was an aggregation of masses stained deeply with Sudan III. Therefore at the periphery where the process of calcification was still continuing there was definite evidence of the presence of fats.

In studying the decalcified sections it was noticed, as frequently observed by others, that the decalcified areas stained more deeply with hæmatoxylin than does normal tissue. Very few nuclei were recognized. The matrix left behind after removing the calcium salts was of a hyaline nature. The existence of this matrix led Litten and others to the theory that the calcium salts are deposited as calcium albuminates. That the fat or soapy substances, still exist in connection with the matrix is indicated by the light-yellow color given with Sudan III; and remembering that, as we have noted, soap solutions and albumins form insoluble compounds with which calcium can combine, it would seem more probable that we are again dealing in this instance with the resultant albuminous material deposited after the calcium has separated from the combination to unite with phosphoric and carbonic acids, and possibly to some extent also after a part of the soap has been split off to enter into more soluble combinations.

Calcified Fibromata.—A similar condition is found in calcified fibroma, in which the most extensive infiltration is not in the centre of the fibroid mass but in the outermost layer, whence it is proceeding towards the centre. In each of the specimens examined, three zones of change were well marked. In the outermost area (which varied in thickness), when the sections are stained with both Sudan III and silver-nitrate solution there is seen a dense black mass, the small amount of soap present in this zone being wholly obliterated by the black-stained lime salts. At the margin of this zone and farther inwards clusters of granules are to be seen, some of which stain with Sudan III, others with silver nitrate. At times an individual cluster, black at one end, yellow at the other, can be made out. In this area, between the groups of granules can still be seen the wavy fibres of connective tissue. This constitutes the second zone. The third zone, farther inwards, exhibits granules staining only with Sudan III. Thus there is well-marked fatty change, the presence of calcium soap, and finally the presence of calcium salts showing no soapy constituent.

That calcifying fibroid tumors should show a deposit of lime salts at the periphery is a phenomenon of the same nature as has been observed in connection with lithopedia and with kidneys undergoing calcification after complete cutting off of their blood supply. The fibroid tumor that undergoes calcification is one that is undergoing necrosis through defective blood supply, and the calcareous salts deposited are not those already present in the tissue itself but those precipitated from the lymph which diffuses into the dead tissue from without. It follows that the diffusible salts are taken up by the first soapy substances with which they come into contact in this process of diffusion, and only when those in the peripheral zone become calcified do the soapy and fatty matters situated more deeply have the opportunity to form calcium compounds.

Calcareous Infiltration of Ovarian and Broad Ligament Cysts.—Cysts of this nature, in which a thin shell of lime salts is laid down in the fibrous tissues of the walls, are occasionally encountered. The process is usually confined to a layer bordering on the cyst cavity beneath the lining membrane of the cyst in the surrounding fibrous tissue. In these we have found the usual evidence of fatty change, of soap formation in the immediate neighborhood of the areas, and of deposition of lime salts.

Calcareous Tubercular Nodules.—The nodules can be decalcified, cut by the freezing microtome, and the sections treated with Sudan III or Scharlach R. It has been usual of late years to speak of tubercular caseation as a process of protoplasmic degeneration in which there is very little associated fatty degeneration. It is interesting therefore to note that by employing the methods described by us it can be demonstrated that the caseous areas which have undergone calcification contain a very considerable amount of fatty matter, so much indeed that the yellow staining areas of the tubercular nodule stand out very prominently from the surrounding tissue. Treatment of such sections with ether removes some of the material stainable with Sudan III, but by no means all. As might be expected from what has already been said, differences are to be made out between old and more recent calcified tubercular areas. In dense, old, calcified glands and nodules there is less of the fatty staining material left, and it is only here and there, where the soap moiety has not been wholly removed, that the deposition of the lime salts gives any results with Sudan III, while in softer and less dense nodules larger and smaller granules staining with Sudan III are abundant. It is noticeable that very little fat is to be made out in the enveloping fibrous tissue. The question naturally arises, Where does this fat come from? Examination of lung tissue in cases of active tuberculosis showed that the fat and its products are already present in tubercles showing the early stages of central necrosis which as yet scarce show caseation. In caseous tubercles this fatty matter is still more evident, and in these also calcareous salts in the form of fine granules are to be detected. It was only in those tubercles in which the fatty substances were aggregated into larger granules that lime salts could be demonstrated. In the earlier non-caseous areas there was no reaction of the calcium; and it is to be noted that there were more Sudan-III-staining granules in the centre of the caseous areas than at the periphery. This series of events indicates the following stages, viz: necrosis, fatty degeneration, formation of calcium soaps, and deposition of calcium salts having no soapy moiety.

Pancreatic Fat Necrosis.—It has been pointed out by various observers that in pancreatic lesions followed by fat necrosis the neutral fats within the fat cells undergo decomposition, and as a result fatty acid crystals are left behind. These fatty crystals after a time unite with the lime salts of the tissue fluids to form insoluble calcium soaps. We found that in the experimental production of fat necrosis the fatty acid crystals could be stained readily enough, but unless the animals experimented upon remained alive for more than a week the lime salts were not to be demonstrated microscopically in the lesions. In other words, the combination of the fatty acids with calcium to form calcium soaps is not an immediate but rather a slow process. A definite period of time seems to be necessary before the calcium soaps accumulate so as to be present in stainable quantities. We examined one specimen of hæmorrhagic pancreatitis in man and found that fatty acid crystals had been deposited in the peripheral zone of the necrotic area. In this zone calcium salts were seen in small amounts distributed as a fine dust. The specimen, however, was an accidental find in a recent autopsy, two small foci of necrosis in the tail of the pancreas, each scarce one centimetre across, being present, and there was no clinical indication of the length of time that the condition had existed. The earlier observations upon the presence of lime soaps in connection with pancreatic fat necrosis will be referred to later.

The preceding section of this paper may be reviewed as follows:

(1) All calcareous infiltrations are preceded by fatty changes and in them substances are found consisting of neutral fats, fatty acids, and soaps, which stain with Sudan III.

(2) By employing appropriate methods, the microscopic fat and fatty acids can be removed, the soaps remaining behind, the last being detected by reason of their differential staining with Sudan III.

(3) The granules in certain regions which give the soap reaction with Sudan III give also the calcium reaction with silver nitrate.

(4) As the process of calcification advances, many of the masses deposited in the part no longer stain with Sudan III, but only react for calcium salts.

In areas undergoing calcification three zones are to be made out: (1) The oldest zone of complete calcification, exhibiting dense calcareous deposit lying in a hyaline matrix; in this zone little soap or fatty substance is to be demonstrated. (2) The intermediate zone, in which granules of lime salts and soap granules are in close apposition; some of these granules give both the calcium and the soap reaction. (3) The most recent zone, which gives the reaction for fat, fatty acids, and soaps and fails to reveal calcium salts in sufficient quantities to be recognized under the microscope.

ON THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PHYSIOLOGICAL CONDITIONS.

Since the observations above detailed indicate that soaps play a most important part in the process of pathological calcareous change, it appears desirable to compare with them observations made upon the part played by the soaps in the physiology of the animal economy.

In 1857 Marcet stated that during fat digestion the fats are converted into soaps in the intestinal canal and these soaps are absorbed by the intestinal mucosa. The absence of any amount of soaps in the blood comparable with the amount of fat absorbed led others to conclude that the soaps as such did not pass beyond the endothelial cells, and to suppose that a glycerin molecule is furnished by these cells, the reconstructed fat being passed into the lacteals. Heidenhain pointed out that some at least of the fat is taken up by the leucocytes on the surface of the intestine and is carried by them directly into the lacteals and blood-vessels. More recently, Ramond has denied the validity of Heidenhain's observations, and has concluded that the greatest part of the fat absorbed enters the portal system and not the lacteals, and enters in the form of soap and not of fat. From this statement we infer that Ramond regards the soap as becoming transformed into fat during its passage from the endothelial cells to the portal capillaries. He finds also that the liver tissue contains a ferment capable of splitting fats into their respective fatty acids. Beneke and later Hoppe-Seyler have pointed out that the blood normally contains soap in solution. Beneke makes an interesting suggestion as to the manner of recovery from pulmonary fat embolism. He points out that possibly a solution of the fat of the emboli is brought about through the action of the lipase present in the blood, which converts the fat into a soluble soap. He also states that fat inoculated subcutaneously disappears so rapidly that the only satisfactory explanation is one based on its conversion into soluble soap.

Still more recently Schultz, who studied fats as a whole in the blood, found that no less than 28 per cent. of the total fatty acid obtainable is present in combination as soap. During starvation, when the fat deposits are used up, he found an increase of the fat in the blood and concluded that the fat is transported from its normal deposits by way of the blood. The fat in the tissue cells he holds becomes transformed into soap and thus passes in solution into the blood.

As to the manner of conversion of the fats into the fatty acid, in which form combination occurs with alkalies to produce soaps, we have the observations of Hanriot. This observer found that blood serum, pancreatic juice, and liver juice contain a ferment capable of converting the neutral fats into their respective fatty acids. This ferment was not present in muscle tissue, testicle, or thyroid gland. The substance isolated by Hanriot seemed by its action to be a true ferment, and was present in all the animals examined. He made a distinction between pancreatic lipase and serum lipase, claiming that they are different substances chemically. Cohnstein and Michaels, who also found a lipolytic ferment in the blood, differ from the former observer in believing that it is present in the red blood cells rather than in the serum.

There can be little doubt that the leucocytes and endothelial cells play parts in the synthesis of fat. It is well known that leucocytes are fat-carriers and that they transport fat from the intestinal canal during fat digestion. The quantity of fat transported must be very small indeed, but Arnold has found that subcutaneous injection of soap solutions into the backs of frogs attracts leucocytes which become filled with fat, and he also noted that eosinophilic leucocytes are especially active in this fat synthesis. Fischler has shown by similar experiments that subcutaneous injections of soap are followed by deposit of fat in the organs, and that when a soap solution is passed through the renal vessels the fat particles can be seen between the endothelial cells and in the kidney tissue. Hence it would seem that endothelial cells take part in the conversion of soap into fat. This view is also supported by the statements of Ramond, namely, that the fat from the intestine passes through the intestinal mucosa, as through a dialyzing membrane, in the form of soap and is reconstructed on entering the portal system.

Munk studied the toxicity of soap and pointed out that soap solutions injected into the portal vascular system are only one-sixth as toxic as when inoculated into the peripheral vessels. From this it would seem that the portal endothelia or the liver parenchyma are especially active in converting the soaps into neutral fats.

THE PRESENCE OF SOAPS IN THE ORGANISM UNDER PATHOLOGICAL CONDITIONS.

It is not a little remarkable that very few observations upon the part played by soaps in pathological conditions have appeared in the literature. We have encountered isolated notes only upon the subject, and do not know a single research, properly so-called, in which their rôle has been fully worked out. Many years ago Virchow called attention to the presence of calcium salts in degenerating lipomata, and very correctly concluded that the fats present in these tumors combine with the calcium salts to form soaps. Kyber in 1880 referred incidentally to the part which both alkalies and fatty acids must play in the process of calcification. Somewhat later Jaeckle and others enunciated the theory that the formation of soaps is one step in the process of calcification of fatty tumors. But this theory has to our knowledge never been tested experimentally and would seem to have become obsolete. In 1890 Langerhans pointed out that in fat necrosis following pancreatic lesions crystals of fatty acids appear, and in many cases unite with calcium of the blood to form an insoluble calcium stearate which occurs at the site of the lesion. It does not appear that Langerhans ever continued these observations or expanded his findings so to apply them to calcification in general. Quite recently Fischler has published a preliminary note upon certain methods for detecting the presence of fats and soaps in the tissues; and though he does not state it definitely, he nevertheless seems to imply that his observations point to an important part played by soaps in more than one process.[1]

As indicated in the preceding pages, our observations all point to the fact that the formation of soaps is a necessary step in the development of pathological calcification. We may at this point present our further studies on the chemistry of this process.

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[1] The number of the *Centralblatt* containing Fischler's article reached America, I may add, after I had forwarded to Philadelphia the abstract of my preliminary communication upon this subject, which was read at the meeting of the Association of American Physiologists in December, 1904. A study of Fischler's paper will show that he has reached his conclusions by the employment of methods differing from those given here.

The analysis of aortas which were in the process of calcification yielded calcium soaps in small quantities together with sodium and potassium soaps, though chemical extraction did not give as clear an idea of the process as was obtained by treating sections with petroleum ether and later with Sudan III. Sections so treated seemed to indicate the presence of a fair amount of soaps. By analysis the quantities obtained were very small. Indeed the Sudan III stain after treatment seemed to show clearly that substances which *in vitro* are soluble are relatively insoluble when present in the tissues. For instance, sodium and potassium soaps are, as we know from every-day experience, easily soluble substances, while, on the contrary, calcium soaps are relatively insoluble. We were prepared, therefore, to find that the tissues contain relatively small amounts of the former, and large amounts of the latter, supposing that when formed locally sodium and potassium soaps would diffuse into the blood and lymph and so escape to a very large extent; but to our surprise the sodium and potassium soaps were not nearly so soluble in tissues as they are *in vitro*—in fact they are relatively insoluble in the former location.

ON THE EXISTENCE OF A COMPOUND OF SOAPS WITH ALBUMEN.

What would seem clearly to be the explanation of this difference was discovered in conducting observations in the test tube. Doubtless the reaction which we are about to describe has been noted before, but, if so, it has failed to come to our attention. If to a weak solution of a pure soap—sodium stearate for example, though the same is true of the oleate and the palmitate—there be added dilute egg albumen, a white rather flocculent precipitate forms. The reaction is not immediate: at the temperature of the room it requires half an hour or longer before it is complete; the precipitate is a combination of soap and albumen. The compound is slightly soluble in water and less soluble in alcohol. Under the microscope the precipitate appears granular, and when the particles are treated with Sudan III they assume the identical pinkish-yellow color of the soap granules observed in tissues undergoing calcification.[1]

It is worth noting that the precipitate becomes denser and more pronounced when carbon dioxide is passed through the solution. Acid calcium phosphate may be added to a solution of egg albumen without precipitation; but if the salt be added to a combination of weak soap solution and egg albumen, made as above, precipitation takes place rapidly and yields a dense sediment.

The Chemical Isolation of Soaps from Pus.—We have already stated that we have been able to isolate *soaps* from more than one order of calcified tissue. We were able to isolate a material soluble in absolute alcohol, insoluble in petroleum ether, and precipitable on the addition of acid calcium phosphate from a number of calcified tuberculous glands of the mesentery. On the addition of a mineral acid the fatty acid was isolated from this combination and gave the characteristic stain with Sudan III.

Particular attention is called to the fact that similar soaps may be present in pus. The cases from which the pus was examined were, it is true, of chronic nature; and no opportunity has yet come to us for the study of large quantities of pus of more acute origin. With the pus of two tubercular abscesses (one an empyema, the other a case of Pott's disease), each yielding fifty cubic centimetres, tests were made. Each quantity was extracted with warm absolute alcohol for seventy-two hours, after which the mixture was filtered and the filtrate evaporated to complete dryness. The residue consisted of fat drops mixed with a white granular substance. The residue was collected and washed with ether and petroleum ether for four days, after which no fat or fatty acid could be demonstrated in the filtrate. It was now digested with warm distilled water, and on the addition of calcium chloride to a quantity of the watery extract a white precipitate, stainable after evaporation on a watch glass with Sudan III, was obtained. In the unstained condition the precipitate appeared as a white fine powder. Fatty acids were obtained from the residue by adding acid, and the addition of hydrochloric acid to the aqueous solution caused an opalescence in the test tube. Sodium and potassium were detected in the residue by the flame, but no calcium was found. Fatty acids could also be detected and saponified from the extract in the petroleum ether.

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[1] Mr. O. R. Mabie, Phm.B., has undertaken a study of this soap-albumen compound. His studies are at the present incomplete, but he has found that solutions of albumen when mixed with known strengths of chemically pure soap solutions absorb a certain quantity of the soap. He has used albumen from different sources, and with all he has obtained similar results, although different degrees of absorption have resulted.

ON THE EXPERIMENTAL PRODUCTION OF CALCIFICATION.

In Capsules Containing Fats and Fatty Acids.—Early in the course of this research experiments were undertaken to determine the organic compounds with which calcium salts may unite. Various products obtained as pure soaps were placed in celloidin capsules prepared by the method recommended by McCrae and introduced into the peritoneal cavity of rabbits where they were left for several days. The results of these experiments led to the study of the rôle of fatty acids and soaps in the process of calcification.

Von Kossa places the normal calcium strength of the blood of rabbits as varying from 1.06 to 2.01 per cent., which may be taken as about the limits of range. In our experiments, two celloidin capsules were prepared containing one gramme of sodium stearate and one gramme of sodium palmitate respectively. These were inserted into the peritoneal cavity of a rabbit and allowed to remain there eleven days. On removal, the contents of the capsule were more or less caked and contained some serum. The contents were treated with hydrochloric acid and precipitated with ammonium oxalate, and the solution then rendered alkaline with ammonia. The precipitate was collected and the calcium determined in the ash obtained on ignition. The sodium stearate was found to have combined with the greater quantity of calcium, there being 4 per cent. of the alkaline earth present. The capsule containing the sodium palmitate was found to contain 3.8 per cent. of calcium. Hence it was found that even in the short space of eleven days a definite conversion of the sodium and potassium soaps into those containing calcium had taken place. Other experiments were carried out in which stearic and palmitic acids were substituted for their salts. The results were similar to the first, there being a decided increase in the calcium strength over that present in the serum of the animal.

The Experimental Production of Calcareous Infiltration in the Kidney.—All cells use fat in some form in carrying out their normal physiological function, and, as has already been pointed out, a great deal of fat is brought to them as soap. When the cell is injured, the soap it would seem is not metamorphosed but remains fixed in the cell in combination with the cytoplasm. This form of union is indicated by experiments on the kidney epithelium in animals, for the main conclusions to be drawn from Litten's experiments are that the tissue cells undergoing calcification are in a degenerating state and have already lost, or are in process of losing, their vitality. He further pointed out that calcification proceeds more rapidly if along with the dead and dying cells there should remain a good circulation to supply the calcium and other materials which are eventually deposited as a permanent precipitate. As Litten examined his sections only with hæmatoxylin staining he found no evidence of fatty products laid down in the areas of his "coagulation necrosis."

The animal experiments reported by Litten were the first dealing with the question of the calcification of the renal tubules. They demonstrated that, if the renal vessels were ligated for two or more hours and then loosened, after eight or ten days crystals of lime salts were to be found in the cells of the convoluted tubules chiefly. The better the blood supply to the organ after ligation, the more quickly did the calcification proceed. The explanation offered was as follows: arrest of the blood to the kidney having caused "coagulation necrosis" of the tubular epithelium, the later advent of the calcium-containing tissue fluids brought about precipitation in the tubules of "calcium albuminate." Although this theory of Litten has been current for twenty years, it is noteworthy that the production of calcium albuminate had not been demonstrated *in vitro*.

We must, however, conclude that in the cells which are injured there is a change in the protoplasm, and of this there is adequate evidence found in the fact that the nuclei stain poorly and the cell substance appears swollen and homogeneous. The alteration of the nucleus is perhaps the chief indication that the cell has suffered grave metabolic changes. There is no evidence at this point of the process that the cell substance is diminished in amount—indeed, on the contrary, the actual amount of cell material appears increased. This is in keeping with the recent observations which point to the conclusion that fat appearing in the cell is not the product of cytoplasmic decomposition, but is absorbed from without. The view we wish to emphasize is that in the degenerating cells absorption of material from without still continues whether this material be albuminous or fatty. But owing to nuclear disturbance, or more broadly to lowered metabolic activity, the substances so absorbed cannot be used up; they no longer become combined into the cell cytoplasm or bioplasm proper, and remaining in an uncombined state may now become subject to various pathological changes.

Neuberger has shown that a number of chemical irritants produce the same effect on the kidney as ligating the renal vessels, and that they lead to calcification of the same sets of tubules. We repeated Neuberger's experiments, using the salts of the heavier metals, mercury, copper, lead, etc., with the result of producing calcareous degeneration in the convoluted tubules of the cortex of the kidney. The rabbits were inoculated subcutaneously with solutions of the soluble salts of these metals and then killed after ten days. A small portion of the kidney was removed for microscopical purposes. Frozen sections were stained with Sudan III and silver nitrate and both gave satisfactory pictures. In consecutive sections it could be shown that the degenerated tubules which reacted for the fatty substances with Sudan III also reacted with silver nitrate; and, moreover, that when a section was treated first with Sudan III and after staining was subjected to silver-nitrate solution, all the Sudan-III-staining tubules were covered over by the intense black stain of the silver salt. That is, the identical tubules which were shown to contain a fatty-staining substance also contained calcium salt (vide Figs. 1 and 2, Plate xxx). The calcium could be wholly removed from the tissue by the use of dilute acids without affecting its fatty-staining qualities. If frozen sections were treated first with seventy per cent. alcohol and later with petroleum ether, it was still possible to find the tubules picked out with Sudan III and silver nitrate. Finally it has been possible to extract from the cortex of the kidney, potassium, sodium, and calcium soaps. These results indicate that the formation of a calcium soap is one stage in the process of calcification in the kidney; that, degeneration and death of the cells being effected, the cells are not able to utilize the potassium and sodium soap brought by the blood, and hence these are precipitated in combination with albumens, and later the calcium from the tissue fluid and blood is fixed in an insoluble state by this compound. While we agree with Litten, Neuberger, von Kossa, and others, that necrobiosis, improperly referred to by them as a "coagulation necrosis," occurs, we look upon this as only one stage in the whole process of calcification.

Von Werra believes that such degenerated cells of the kidney may return to a normal condition. We can hardly accept this view, as calcareous degeneration of cells is the last stage in a pathological process in which the cells and nuclei are disintegrated. Hence it is improbable that they can regain their vitality.

Tartarini-Galleani claims to have studied the kidneys of rabbits after injecting bichloride of mercury into the substance of the organ. He states that the lesion produced is a tissue destruction followed by fibrinous coagulation, which after the ninth day becomes impregnated with calcium salts. The calcification in the kidney remains circumscribed to the area of the organ directly affected by the injection, and he holds that it is neither preceded nor accompanied by the phenomenon of fatty change. We have repeated his experiments in two rabbits and in each the result was different from that described by him.

Sections of the inoculated kidney treated with Sudan III showed the cells of the convoluted tubules to be swollen and filled with fatty granules. The tubules were well outlined from the surrounding tissue by reason of the stained fat in the cell contents. The glomeruli and the interstitial tissue appeared unchanged, while the arterioles of the cortex showed Sudan-III-stained granules in their walls. These granules were distributed more loosely than was seen in the epithelium. The affected convoluted tubules presented collections of irregular granules staining not so intensely as neutral fat, but rather a brownish-yellow. The tubules in the pyramids showed a striking difference. While they were less affected than in the cortex the condition of the fat in them differed from that of the convoluted tubules. The individual cells showed no change in size or shape, and the nuclei retained their staining power. However, the Sudan III picked out numerous fine globules situated towards the inner margins of the cells—that is, towards the lumen and away from the nucleus. In some cases it appeared that the fatty globules were in the process of being extruded into the lumen. The Sudan III granulation noted in the straight tubules was of the character of neutral fat. No calcium deposit could be demonstrated in the straight collecting tubules, while in the cortex the convoluted tubules which showed the Sudan III granulations reacted to silver nitrate in the manner of calcium salts.

Résumé.—Our studies upon experimental calcification in the kidney confirm and extend the observations made upon calcareous changes in human tissues. If the tubules become the seat of the calcareous deposit it is possible to recognize the following stages, viz:

(1) A stage of cell degeneration characterized by swelling of the cell substance and diminution of the nuclear chromatin, the cells becoming swollen and homogeneous;

(2) A later stage in which fat appears in the cells, apparently having arisen by absorption.

(3) A final stage in which calcareous salts appear in the cells accompanied by soaps. An intimate relation between cytoplasmic degeneration and the formation of insoluble soaps would seem to exist. Judging from observations made in test tubes,

the relatively insoluble soaps are compounds between soap and certain albuminous matters liberated from the cytoplasm. The calcium from the tissue fluids and the blood becomes fixed by the albumen-soap compound.

Experimental Calcification of Muscle Fibres.—That muscle fibres may become the seat of calcareous deposits is well known. We inoculated a weak solution of bichloride of mercury into the quadriceps muscle of a rabbit, killing the animal at the end of eight days. On examination the muscle at the site of the inoculation showed extensive degeneration and softening. Individual fibres were swollen and in some places the sarcolemma was broken. Other swollen fibres showed band-like constriction. Still other muscle fibres were completely broken down and invaded by numerous polymorphonuclear leucocytes.

The degenerated muscle fibres proved an interesting study. A fine granulation not found in healthy fibres, and staining in Sudan III, occurred in their substance, besides which leucocytes loaded with fat globules invaded the degenerating fibres and lay in the interstitial tissue. The fatty-staining material within the leucocytes appeared in the form of large and small granules, but these did not coalesce to form large fat globules as is seen in adipose tissue.

The question arises as to whether the leucocytes are bringing the fat to the injured tissue or taking it away. We are inclined to the latter view, for among the degenerated fibres were some in which the sarcolemma was intact and which were free from leucocytic infiltration, but which nevertheless contained granules staining with Sudan III. Moreover, the leucocytes at the margin of the injured tissue contained no fatty-staining material.

We think it probable that this process is not unlike that described in the kidney, namely, a state of lowered vitality of the individual cells through which, being no longer able to deal with the fatty material brought to them in solution, they form with it a relatively insoluble compound in the cytoplasm of the degenerating cells.

Experimental Fat Necrosis.—In order to investigate further the changes which fatty products undergo in the body, we produced fat necrosis in a rabbit by the intraperitoneal inoculation of fresh extract of pancreas. The injection was repeated on the second day and the animal killed on the fifth day. At the post-mortem a few tags of fat necrosis about the size of a pea were found scattered in the mesentery and the posterior abdominal wall. Frozen sections of these showed that the fat did not exist in the large distended fat cells but that the globules were replaced by numerous fine crystalline particles staining with Sudan III. On treating the sections with silver nitrate, the subperitoneal margins of the fat necrosis showed the presence of a slight amount of calcium salts appearing like a fine dust-like deposit. All the areas of fat necrosis did not show the presence of calcium with silver nitrate

As already stated, Langerhans showed in 1890 that calcium salts appear in the areas of fat necrosis. He describes the process as one in which the fat is converted by ferment action into fatty acid. The calcium salts of the blood then become attracted to the fatty-acid moiety and form a calcium soap.

ON THE SOURCE OF THE FAT IN THE DEGENERATED CELLS.

Before concluding, it is, we think, essential to indicate our views regarding the source of the fat present thus in all cells that undergo calcification and essential for the due development of the process. To discuss the matter adequately would demand that the numerous recent studies upon fatty degeneration be passed in review: such full discussion would carry us too far afield. It must suffice that we call to mind the reasonable deductions from the work of recent observers which appear to bear immediately upon our present problem.

We may, I think, in the first place safely accept that fats are absorbed by the cell from the surrounding medium in the form of diffusible soaps. Taken up by the cell these soaps may either be reconverted into neutral fats and stored as such in the cytoplasm, or may undergo assimilation proper, becoming part and parcel of the cell substance. That such assimilation does occur with associated loss of individuality on the part of the fats is, we hold, demonstrated by sundry recent observations. Thus Rosenfeld and others have shown that kidneys presenting no fat recognizable by microchemical means will, nevertheless, yield upon careful analysis as much as fifteen per cent. of fats; and Doremeyer, that mere extraction by ether will not liberate the greater part of tissue fats: the cells have to be digested with pepsin before all can be isolated; a clear indication that the fats have previously been in combination with proteid substances in the cells.

If, then, these possibilities be accepted, it follows that the appearance of fats and soaps in a degenerating cell may be from at least two sources, namely:

(1) The absorption of soaps from the circulating lymph and blood, the cell still retaining the power of splitting off the fat, but being unable to utilize this in metabolism, so that it becomes stored up in a non-diffusible form.

(2) Disintegration of the cell substances so that the fats become liberated from their combination with the cytoplasm and appear in the form of discrete particles, or globules, of neutral fat, which now reveal their presence by reacting with Sudan III.

A third possibility has to be kept in mind, namely, that soaps may be absorbed from the blood and lymph by cells with vitality so depressed that no conversion takes place of the absorbed soap into neutral fat, but combining with the disintegrated albuminous molecules this forms a relatively insoluble soap-albumen compound.

Into the controversy regarding the formation of fat by the decomposition of the albuminous molecule I will not enter, save by suggesting that the opposing views may possibly be harmonized through the demonstration here afforded, that albumens form a combination with soaps. The increase in fats determined in tissue autolysis by Waldvogel, Hildesheim and Leathes, Landsteiner and Mucha, and others is, it may be, to be explained, not as due to the appearance of a decomposition product of the albumen molecule proper, but as brought about by dissociation of this albumen-soap compound.

The time is not ripe for, neither do our personal observations up to the present permit, any discussion of the part played by cell ferments in any of these processes.

As to which of the above alternatives—absorption or dissociation—is the more frequently in action as a precursor of calcification, we would point out that when the cell becomes distended with fatty globules (and this is common in atheromatous areas) we must conclude that absorption from without is in the main responsible for the condition.

CONCLUSIONS.

It will be seen from the above that we have studied the conditions associated with the deposit of calcareous salts: (1) in connection with normal and pathological ossification, and (2) in pathological calcification as exhibited in (a) atheroma of the vessels; (b) calcification of caseating tubercular lesions; (c) calcification of inflammatory new growth, and (d) degenerating tumors; and we have induced experimentally deposits of calcareous salts in the lower animals: (a) within celloidin capsules containing fats and soaps; (b) in the kidney, and (c) in connection with fat necrosis.

I. We have found that bone formation and pathological calcareous infiltration are wholly distinct processes. In the former there is no evidence of associated fatty change, and the cells associated with the process of deposition of calcium are functionally active. In the latter there is an antecedent fatty change in the affected areas, and the cells involved present constant evidences of degeneration. The view that would seem to account best for the changes observed in the latter case is that with lowered vitality the cells are unable to utilize the products brought to them by the blood, or which they continue to absorb, so that the normal series of decompositions associated with their metabolism fails to take place and hence they interact among themselves in the cytoplasm with the result that insoluble compounds replace soluble ones.

II. Besides the fact that calcification is always preceded by fatty change within the cells, another fact should be emphasized, namely: that combination of the fats present with calcium salts to form calcium soaps tends to occur. The stages immediately preceding these are difficult to follow with anything approaching certainty, perhaps because the earlier stages vary under different conditions. In fat necrosis, for instance, the cells affected are normally storehouses for neutral fats, and as long as they remain healthy neutral fats alone are present in them. When they are subjected to the action of the pancreatic juice with its fat-splitting ferment the cells are killed and coincidently the neutral fats are decomposed, fatty acids being deposited. The fatty acids now slowly combine with the calcium salts. In degenerating lipomata the process would seem to be similar.

But in other cases the cells are not obviously fat-containing in the normal state; nevertheless prior to calcification they undergo so-called fatty degeneration, which is really a form of cell degeneration accompanied by fat infiltration. As regards the source of the cell fats in general we may safely accept:

1. That fats are transported in the blood as diffusible soaps.
2. That taken up by the cells these soaps may either—
    - (a) Be reconverted into neutral fats and become stored in the cytoplasm as such, or
    - (b) undergo assimilation proper, becoming part and parcel of the cell substance, in which case they are not recognizable by the ordinary microchemical tests.

3. If these two possibilities be accepted it follows that the appearance of fats and soaps in the degenerating cell may be due to either—

    - (a) Absorption or infiltration of soaps from the surrounding medium, the degenerating cell retaining the power of splitting off the fat but being unable to utilize this in metabolism.
    - (b) Cytoplasmic disintegration with dissociation of the soap-albumen combination or, more broadly, liberation of the fats from their combination with the cytoplasm.

The appearances seen in the cells of atheromatous areas indicate that the first of these does occur.

III. In areas undergoing calcareous infiltration we have demonstrated the presence of soaps, and this often in such quantities that they can be isolated and estimated by gross chemical methods. By microchemical methods also we have been able to show that after removing all the neutral fats and fatty acids by petroleum ether there remains behind a substance giving with Sudan III the reaction we associate with the presence of soap. And experimentally we have produced these soaps within the organism, more particularly by placing capsules containing fats and fatty acids within the tissues and after several days finding that the capsules contain calcium soaps and possess a calcium content far in excess of that of the normal blood and lymph.

IV. While these are the facts, certain of the details of this reaction demand elucidation. The existence of sodium and it may be potassium soaps in the degenerated cells is comprehensible if we accept that these are present in the circulating lymph and simply undergoing absorption. But even then, as these are diffusible substances how is it to be explained that they become stored up in these particular areas?

We have found that, as a matter of fact, in regions which give the reaction for soaps, but which give no reaction for calcium (which therefore presumably contain at most amounts of the insoluble calcium soap too small to need consideration), the ordinary solvents for potassium and sodium soaps do not forthwith remove the stainable material; they are relatively insoluble.

The reason for this insolubility is suggested by the observations made in the test tube, that soap solutions mixed with solutions of white of egg or blood serum form a precipitate of combined soap and albumen, which likewise is insoluble in water and alcohol. The indications are therefore that in cells undergoing degeneration, with degeneration of the cytoplasm, certain albuminous molecules unite with the soaps present to form relatively insoluble soap-albuminate.

V. With regard to calcium soaps, these are also present and in certain stages appear to be the dominating form in the affected tissues. Two questions suggest themselves, viz.: what is the source of calcium, and what is the process by which they become formed?

As to the source, the amount present in well-marked calcification is far in excess of the normal calcium contents of the affected tissue. If in the kidneys of experimental calcification three hundred times as much calcium may be present as in the normal kidney (von Kossa), the calcium must be conveyed to the part by the blood and lymph, and that this is so is demonstrated, as we have pointed out, by the distribution of the infiltration in solid organs, that like ovarian fibroids have undergone necrosis, in which the earliest deposits are superficial.

As to the process, there are three possibilities:

1. That sodium and potassium soaps and soap albuminates are first formed and that interaction occurs between them and the diffused calcium salts from the lymph, the less soluble-calcium replacing the sodium and potassium.

2. That under certain conditions the calcium salts act directly on the neutral fats present in the degenerating cells.

3. That the neutral fats are first broken down into fatty acids and that these react with the calcium salts to form the soaps.

We are assured that the first process occurs and that because in the boundary zone of areas of calcification we can detect soapy particles devoid of calcium, identical in position and arrangement with the particles more deeply placed which give the calcium reactions. But this is not the only reaction. In case of fat necrosis we see clearly that the third process is in evidence. And we are far from being convinced that the second does not also obtain. We have been impressed by the large accumulation of neutral fats in the cells in cases of early atheroma and the absence at any stage of the process of recognizable fatty acid. While soaps, it is true, are compounds of fatty acids with alkalies, it is recognized in ordinary domestic life that they can be formed by the direct action of strong lye upon ordinary fats, and this even in the cold. It is quite possible therefore that there occurs a similar direct process in the organism.

The point is worth noting, however, that this does not occur in healthy cells the seat of fatty infiltration. We therefore leave this an open question, only laying down that, as indicated by the hyalin albuminous matrix left when calcium salts are dissolved out of an area of calcification, there must exist a calcium soap- or fat-albuminate similar to the potassium and sodium soap-albuminates already mentioned—such an albuminate as we can form with calcium soaps in the test tube.

VI. In old areas of calcification soaps are largely if not entirely wanting, although these are to be detected at the periphery, when the process is still advancing. The reactions given by these older areas are almost entirely those of calcium phosphate, though some calcium carbonate is at times to be made out.

This seems surely to indicate that the final stage in calcification is an interaction between the calcium soap-albuminates and substances containing phosphoric and carbonic acids. Such substances, it is needless to say, are present in considerable amounts in the lymph and blood. We must conclude that the acid sodium phosphates of the lymph act on the calcium soap, the highly insoluble calcium phosphates being formed (plus the albuminous moiety of the original compound) and diffusible sodium soap being liberated, while similarly alkaline carbonates form calcium carbonate and liberate sodium and potassium soaps. Calcium phosphate and calcium carbonate thus become the insoluble earthy salts of old crystalline areas of calcification.

VII. As already stated very little soap is to be found in these old areas. It is possibly worth suggestion that the soaps liberated in this last reaction, as they diffuse out, again react with diffusible calcium salts and form calcium soaps which once more react with the alkaline salts to produce the phosphates and carbonates; that, in short, they have a katalytic action. Certain it is that old calcareous areas are extraordinarily dense, and have a coarse crystalline structure, wholly at variance with the finely granular appearance of the more recent areas, and these large crystalline masses, it would seem, can only be formed by successive deposition of new material and eventual fusion, as the interspaces become filled in between the original masses.

In conclusion I wish to thank Professor R. F. Ruttan of McGill University and Mr. J. R. Roebuck, B.A., for their kind assistance in carrying out some of the chemical analyses, and Drs. M. E.

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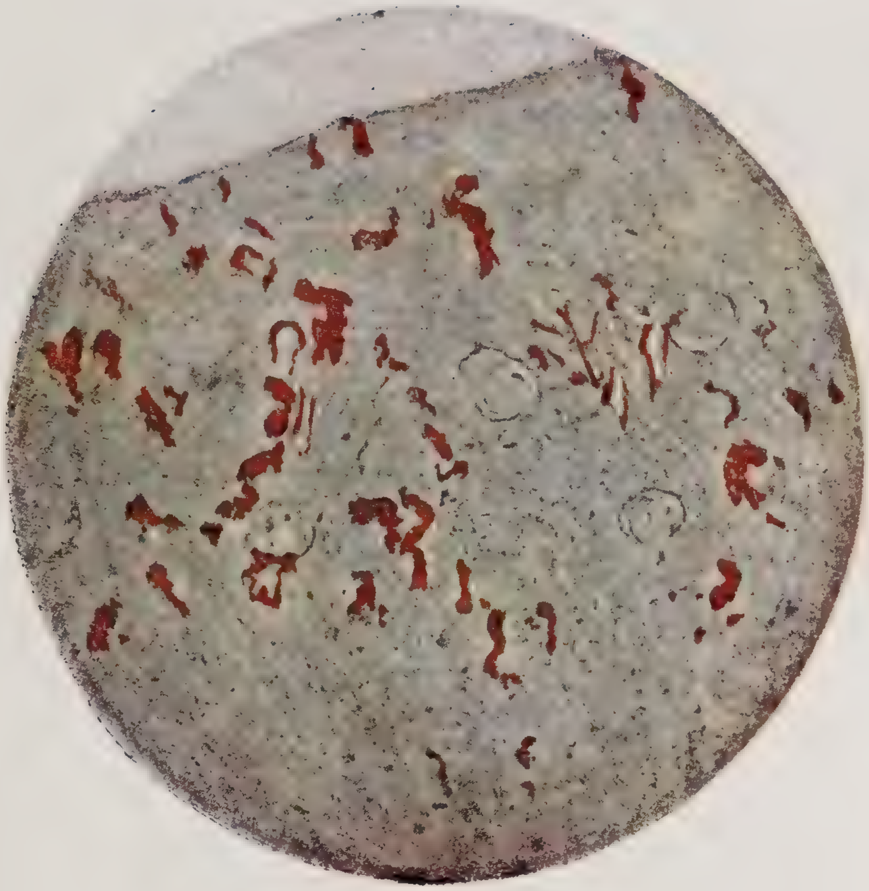

FIG. 1.

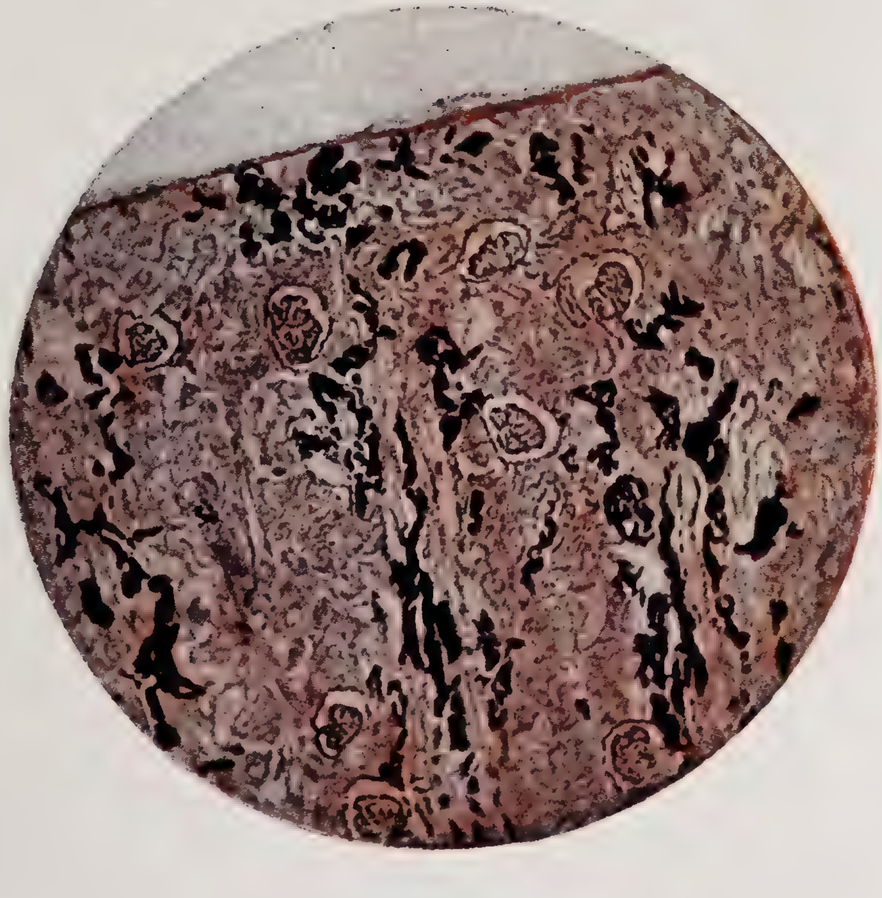

FIG. 2.

DESCRIPTION OF PLATES.

Plate XXX.

Fig. 1.—Kidney from rabbit which died eight days after inoculation with corrosive sublimate. Frozen section stained with Sudan III showing the fatty condition of certain convoluted tubules.

Fig. 2.—Section from the same kidney as Fig. 1, stained with silver nitrate followed by weak saffranin. Similar convoluted tubules picked out by the silver deposit as seen in Fig. 1 with Sudan III.

Plate XXXI.

Fig. 3.—Aorta with large fat masses towards the lumen with similar lighter staining particles near the boundary zone between the intima and media. In the media the blackened deposit representing calcium salts is very intense and is arranged in parallel rows. These rows with higher magnification are seen to be composed of numerous small crystals. Among these rows of calcium salts are also seen the yellowish granules of Sudan III staining.

Fig. 4.—Section of aorta stained with Sudan III. The fatty plaque shows a lighter stained part in its middle, representing the area of soap formation and calcium deposit.

Fig. 5.—Same aortic plaque as seen in Fig. 4, treated with silver nitrate solution. The area which with Sudan III stained a light yellow is now represented as the black deposit, the site of the calcium salts.

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